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☐ 1. Document ID: US 20010020098 A1

L3: Entry 1 of 84

File: PGPB

Sep 6, 2001

DOCUMENT-IDENTIFIER: US 20010020098 A1

TITLE: Cyanine dyes and synthesis methods thereof

DETX:

[0067] Streptavidin (15 mg) is dissolved in 1 mL 0.1M sodium phosphate buffer, pH 7.0. Sulfosuccinimidyl 4-[N-maleimidomethyl] cyclohexane-1-carboxylate (9 mg) in 0.2 mL dimethylsulfoxide is added to the streptavidin solution at room temperature, mixed for 1 hour and purified by gel filtration chromatography to give maleimide derivatized streptavidin. To a 1.5 mL solution of maleimide derivatized streptavidin (3.5 mg) in 0.1M sodium phosphate, 5 mM EDTA buffer, pH 6.0, 1 mg of Cyanine 3 Dye (15) is added followed by 0.15 mL of 1M hydroxylamine pH 7.0. The solution is mixed at room temperature for 1 hour and then 20 .mu.L of 0.1M N-ethylmaleimide is added and the mixture is stored at 4.degree. C. for 16 hours. The mixture is purified by gel filtration chromatography to give streptavidin modified with Cyanine 3 Dye (15).

DETX:

[0068] Streptavidin (15 mg) is dissolved in 1 mL 0.1M sodium phosphate buffer, pH 7.0. Sulfosuccinimidyl 4-[N-maleimidomethyl] cyclohexane-1-carboxylate (9 mg) in 0.2 mL dimethylsulfoxide is added to the streptavidin solution at room temperature, mixed for 1 hour and purified by gel filtration chromatography to give maleimide derivatized streptavidin. To a 1.5 mL solution of maleimide derivatized streptavidin (3.5 mg) in 0.1M sodium phosphate, 5 mM EDTA buffer, pH 6.0, 1 mg of Cyanine 3 Dye (16) is added followed by 0.15 mL of 1M hydroxylamine pH 7.0. The solution is mixed at room temperature for 1 hour and then 20 .mu.L of 0.1M N-ethylmaleimide is added and the mixture is stored at 4.degree. C. for 16 hours. The mixture is purified by gel filtration chromatography to give streptavidin modified with Cyanine 3 Dye (16).

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw Desc	Image
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☐ 2. Document ID: US 6287765 B1

L3: Entry 2 of 84

File: USPT

Sep 11, 2001

DOCUMENT-IDENTIFIER: US 6287765 B1

TITLE: Methods for detecting and identifying single molecules

BSPR:

In another preferred embodiment providing advantages over single-molecule affinity selection, a selected target (e.g., alkaline phosphatase; AP) is immobilized to the silicon nitride AFM probe tip and used to probe a random-sequence, nanosphere-conjugated RNA library applied in solution (i.e., freely diffusible) to a freshly cleaved mica substrate. On detection of aptamer binding to the probe tip, varying unloading (i.e., discharge) forces is applied to dissociate aptamer-nanosphere conjugates from tip-immobilized AP. Individual, high-affinity aptamers are selected on quantitative grounds based on the empirical binding and unbinding forces accompanying aptamer-target association and dissociation from tip-immobilized target. Selected aptameric nucleotides, i.e., those displaying a binding force exceeding an operator-established set point (in micronewtons, relative force or loading and unloading force(s) or force curves relative to a reference ligand-receptor pair such as peroxidase-antiperoxidase, fluorescein-antifluorescein or DNP-antiDNP). In a particularly preferred mode of operation, a panel of reference ligand-receptor pairs having predetermined apparent affinities (e.g., by Scatchard analysis using labeled ligand(s) and a family(ies) of unlabeled competitors) are used to establish a (force x apparent affinity) calibration curve against which the apparent affinity of individual identified aptamer-target pairs can be interpolated from loading and unloading AFM force data. In this way, an individual aptamer having the highest measured binding force can be selected against reference apparent affinities determined for nonaptameric ligand receptor pairs. For example, a family of biotin congeners (e.g., biotin, imidobiotin, diimidobiotin, iminobiotin) can be selected to represent a broad dynamic range of affinities for avidin, streptavidin and/or recombinant or otherwise modified streptavidin and/or avidin mutants. A panel of biotin derivatives or an array of mutant and/or modified streptavidins permuted against biotin derivatives can be used to calibrate the binding force of an aptamer-target complex over a range spanning many decades of apparent affinity (i.e., as related to inverse concentration on log scale).

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw Desc	Image
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☐ 3. Document ID: US 6274322 B1

L3: Entry 3 of 84

File: USPT

Aug 14, 2001

DOCUMENT-IDENTIFIER: US 6274322 B1

TITLE: Composition for introducing nucleic acid complexes into higher eucaryotic cells

BSPR:

The invention also relates to a transfection kit, comprising a carrier means having in close confinement therein one or more container means, wherein a first container means contains a biotin-modified endosomolytic agent and a second container means contains a streptavidin-modified substance having affinity for nucleic acid.

DEPR:

The coupling of streptavidin to polylysine was effected using the method described by Wagner et al., 1990, and in EP-A1 388 758. 79 nmol (4.7 mg) of streptavidin in 1 ml of 200 mM HEPES pH 7.9 and 300 mM NaCl were treated with

a 15 mM ethanolic solution of SPDP (236 nmol). After 1.5 hours at ambient temperature the modified protein was gel filtered over a Sephadex G-25 column, thereby obtaining 75 nmol of streptavidin, modified with 196 nmol of dithiopyridine linker. The modified protein was reacted under an argon atmosphere with 3-mercaptopropionate-modified polylysine (75 nmol, average chain length 290 lysine monomers, modified with 190 nmol mercaptopropionate linker) in 2.6 ml of 100 mM HEPES pH 7.9, 150 mM NaCl. Conjugates were isolated by cation exchange chromatography on a Mono S HR5 column (Pharmacia). (Gradient: 20-100% buffer B. Buffer A: 50 mM HEPES pH 7.9; buffer B: buffer A plus 3 M sodium chloride). The product fraction eluted at a salt concentration of between 1.2 M and 1.7 M. Dialysis against HBS (20 mM HEPES pH 7.3, 150 mM NaCl) resulted in a conjugate consisting of 45 nmol of streptavidin and 53 nmol of polylysine.

DEPR:

For formation of adenovirus-DNA-transferrin complexes 50 .mu.l of biotinylated adenovirus were incubated with 400 ng of streptavidin-modified polylysine in 20 .mu.l HBS for 20 min. Then 20 .mu.l of HBS containing 6 .mu.g pCMVL were added. After an incubation period for 20 min 7 .mu.g of mouse transferrin-polylysine conjugate (mTfpL) in 160 .mu.l HBS were added and the whole mixture was incubated for further 20 min.

DEPR:

The complexes were prepared as follows: 200 .mu..mu.l biotinylated Adenovirus is dl312 diluted with 200 .mu.l HBS were incubated with 6.4 .mu.g streptavidin-modified polylysine in 400 .mu.l HBS for 30 minutes at room temperature. Then 48 .mu.g of pCMV-L in 800 .mu.l HBS were added. After 30 minutes of incubation 48 .mu.g of TfpL in 900 .mu..mu.l HBS were further added. For application of the complexes male Sprague Dawley rats (250 g body weight) were anesthetize with Avertin and the abdomen opened with a median incision. The intestine was displaced to the left side of the body and a 27 G needle, which had been attached to a tube and a 1 ml syringe was inserted into the bile duct. The injection of the complexes was performed over a period of 4 minutes. Then the needle was retracted from the bile duct and the injection site sealed with a fibrin sealer (Immuno). The abdominal wound was closed with sutures and metal clips. After 30 hours the rat was killed and samples from different lobes of the liver were assayed for luciferase gene expression. The pea activity of luciferase was 19000 light units/mg protein and the calculated overall expression in the total liver was in the range of 2.7.times.10.sup.6 light units.

DEPR:

Human skin biopsies were put into a 6 cm petri dish containing DMEM, 2 mM glutamine, 20% FCS and antibiotics. Then the biopsies were thoroughly minced with a surgical knife and cultured in the presence of 3 ml medium for 5 days. Thereafter the cells were washed with fresh DMEM containing 2 mM glutamine 10% FCS and antibiotics and cultured for further 7 days. After this period of time the cells were trypsinized and subcultured into new petri dishes. When the cells were almost confluent, they were trypsinized again and stored frozen until transfection. For transfection the cells were thawed and seeded into 6 cm petri dishes and cultured in DMEM containing 2 mM glutamine 10% FCS and antibiotics. The transfection conjugates were prepared as follows: 3 .mu.l, 10 .mu.l, 20 .mu.l and 30 .mu.l of biotinylated adenovirus dl312 were incubated with 0.1 .mu.g, 0.3 .mu.g, 0.5 .mu.g and 0.8 .mu.g polylysine-modified streptavidin in 150 .mu.l HBS for 30 minutes at room temperature. Then 6 .mu.g of pCMV-.beta.gal plasmid in 170 .mu.l HBS were added and the mixture was incubated for further 30 minutes. In the final step 7.8 .mu.g TfpL for the conjugates with 3 .mu.l dl312, 7 .mu.g TfpL for 10 .mu.l dl312 and 6 .mu.g TfpL for the conjugates with 20 .mu.l and 30 .mu.l dl312 in 170 .mu.g HBS were added. After an incubation period of 30 minutes the conjugates were applied to the cells in 2 ml DMEM containing 2 mM glutamine, 2% FCS and antibiotics and the cells were incubated for 4 hours at 37.degree. C. Then the medium was removed and culture was continued at 37.degree. C. with DMEM containing 2 mM glutamine, 10% FCS and antibiotics. After 48 hours the expression of .beta.-galactosidase was demonstrated as described in previous Examples.

CLPR:

4. A transfection kit, comprising a carrier means having in close confinement therein one or more container means, wherein a first container means contains a biotin-modified endosomolytic agent and a second container means contains a streptavidin-modified substance having affinity for nucleic acid.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 4. Document ID: US 6261554 B1

L3: Entry 4 of 84

File: USPT

Jul 17, 2001

DOCUMENT-IDENTIFIER: US 6261554 B1

TITLE: Compositions for targeted gene delivery

BSPR:

For many cell type-specific antigens protein ligands have been identified which bind with high specificity and/or affinity, e.g. cytokines binding to their cellular receptors. Most proteins can be labeled with the water-soluble vitamin biotin, using simple procedures. Most biotinylations are performed using succinimide esters of biotin. Binding to the protein proceeds through free amino groups, normally of lysyl residues. Biotinylated molecules are bound with exceptionally high affinity ($K_a=10^{14}$ - 10^{15} M⁻¹) by both Avidin and Streptavidin (Wilchek and Bayer, Immunol. Today 5(1984): 39). Avidin is a 68 kD glycoprotein isolated from egg white and Streptavidin is a 60 kD protein from *Streptomyces avidinii*. Both molecules are homotetramers; each subunit contains a single biotin binding-site. The high affinity of their binding makes the biotin-avidin or biotin-streptavidin interactions essentially irreversible. A functional streptavidin gene has been cloned (Sano and Cantor, Proc. Natl. Acad. Sci. USA 87(1990): 142) and a streptavidin mutant has been generated (Sano and Cantor, Proc. Natl. Acad. Sci. USA 92(1995): 3180) with reduced biotin-binding affinity (approx. $K_a=10^8$ M⁻¹) providing specific and tight, yet reversible biotin-binding. As for Protein A and G, avidin and streptavidin do not intrinsically bind to mammalian cell surfaces or virus membranes or capsids. Expression of (parts of) these molecules on the surface of gene delivery vehicles will therefore also require the generation of hybrid molecules. Hybrids of streptavidin and heterologous proteins have been made and retained full biotin binding capacity (Sano and Cantor, Bio/Technol. 9(1991): 1378; Sano et al., Proc. Natl. Acad. Sci. USA 89(1992): 1534).

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 5. Document ID: US 6248539 B1

L3: Entry 5 of 84

File: USPT

Jun 19, 2001

DOCUMENT-IDENTIFIER: US 6248539 B1

TITLE: Porous semiconductor-based optical interferometric sensor

DEPR:

To rule out the possibility of nonspecific interaction, a non-biotinylated surface was subjected to the same solution, and conditions as described in Example 4. No measurable change in the effective optical thickness was observed on treatment with streptavidin, secondary antibody, primary antibody, and digoxigenin. Detection of the relatively small biotin molecule (MW=244) at concentrations as low as 10^{-12} M has also been demonstrated using biotin-streptavidin-modified porous Si.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 6. Document ID: US 6235488 B1

L3: Entry 6 of 84

File: USPT

May 22, 2001

DOCUMENT-IDENTIFIER: US 6235488 B1

TITLE: Surface preparation for chemical-specific binding

CLPR:

2. The method of claim 1 wherein the ligand-binding substance is selected from the group consisting of avidin, streptavidin, acetylated avidin, acetylated streptavidin, succinylated avidin, succinylated streptavidin, genetically engineered avidin with intact biotin binding sites, genetically engineered streptavidin with intact biotin binding sites, modified avidin with intact biotin binding sites, and modified streptavidin with intact biotin binding sites; and said ligand-bearing substance is a biotinylated compound.

CLPR:

5. A device as in claim 4 wherein said ligand-binding substance is selected from the group consisting of avidin, streptavidin, acetylated avidin, acetylated streptavidin, succinylated avidin, succinylated streptavidin, genetically engineered avidin with intact biotin binding sites, genetically engineered streptavidin with intact biotin binding sites, modified avidin with intact biotin binding sites, and modified streptavidin with intact biotin binding sites; and said ligand-bearing substance is a biotinylated compound.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 7. Document ID: US 6225455 B1

L3: Entry 7 of 84

File: USPT

May 1, 2001

DOCUMENT-IDENTIFIER: US 6225455 B1

TITLE: Constructs for producing phosphorylated fusion proteins

BSPR:

The invention also provides particularly interesting labellable and labelled proteins like phosphorylated antibodies (especially monoclonal antibodies, hybrid antibodies, chimeric antibodies or modified antibodies), hormones, and "modified" streptavidin. The modified streptavidin can be bound to individual biotinylated antibodies, each streptavidin being modified by single or multiple phosphorylated groups, which product has greatly enhanced radiation and therefore diagnostic and therapeutic potential.

DEPR:

A particularly noteworthy and interesting application made possible by the invention is what has been called here in the vernacular, a therapeutic or more specifically an antitumor "therapeutic radiation bomb". Such a biologically-active composition uses biotin coupled to a tumor-specific monoclonal antibody (Mab) (or to Fab or Fab' fragments if more appropriate), and a multiple "modified" streptavidin bound to each Mab-bound biotin, each streptavidin being modified in that it has multiple phosphorylated groups. Since streptavidin is itself a tetramer, multiple radioactive groups are thus provided. These multiple radioactive groups expose the tumor with radiation which is greatly amplified and hence more readily detectable and would produce greater tumor destruction. In the case where it is highly phosphorylatable it is much more easily detectable. Thus, each one of the biotins which is bound to each tumor-specific Mab binds tightly to the multiple streptavidin molecules which in turn contain multiple labelled phosphorus atoms, or their equivalent isotopes.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 8. Document ID: US 6225061 B1

L3: Entry 8 of 84

File: USPT

May 1, 2001

DOCUMENT-IDENTIFIER: US 6225061 B1

TITLE: Systems and methods for performing reactions in an unsealed environment

ORPL:

Reznik et al., A streptavidin mutant with altered ligand-binding specificity, Proc. Natl. Acad. Sci. USA 95:13525 (1998).

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 9. Document ID: US 6224644 B1

L3: Entry 9 of 84

File: USPT

May 1, 2001

DOCUMENT-IDENTIFIER: US 6224644 B1

TITLE: Cyanine dyes and synthesis methods thereof

DEPR:

Streptavidin (15 mg) is dissolved in 1 mL 0.1M sodium phosphate buffer, pH 7.0. Sulfosuccinimidyl 4-[N-maleimidomethyl] cyclohexane-1-carboxylate (9 mg) in 0.2 mL dimethylsulfoxide is added to the streptavidin solution at room temperature, mixed for 1 hour and purified by gel filtration chromatography to give maleimide derivatized streptavidin. To a 1.5 mL solution of maleimide derivatized streptavidin (3.5 mg) in 0.1M sodium phosphate, 5 mM EDTA buffer, pH 6.0, 1 mg of Cyanine 3 Dye (15) is added followed by 0.15 mL of 1M hydroxylamine pH 7.0. The solution is mixed at room temperature for 1 hour and then 20 .mu.L of 0.1M N-ethylmaleimide is added and the mixture is stored at 4.degree. C. for 16 hours. The mixture is purified by gel filtration chromatography to give streptavidin modified with Cyanine 3 Dye (15).

DEPR:

Streptavidin (15 mg) is dissolved in 1 mL 0.1M sodium phosphate buffer, pH 7.0. Sulfosuccinimidyl 4-[N-maleimidomethyl] cyclohexane-1-carboxylate (9 mg) in 0.2 mL dimethylsulfoxide is added to the streptavidin solution at room temperature, mixed for 1 hour and purified by gel filtration chromatography to give maleimide derivatized streptavidin. To a 1.5 mL solution of maleimide derivatized streptavidin (3.5 mg) in 0.1M sodium phosphate, 5 mM EDTA buffer, pH 6.0, 1 mg of Cyanine 3 Dye (16) is added followed by 0.15 mL of 1M hydroxylamine pH 7.0. The solution is mixed at room temperature for 1 hour and then 20 .mu.L of 0.1M N-ethylmaleimide is added and the mixture is stored at 4.degree. C. for 16 hours. The mixture is purified by gel filtration chromatography to give streptavidin modified with Cyanine 3 Dye (16).

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 10. Document ID: US 6217869 B1

L3: Entry 10 of 84

File: USPT

Apr 17, 2001

DOCUMENT-IDENTIFIER: US 6217869 B1
TITLE: Pretargeting methods and compounds

DEPR:

Biotin binding capacity of the PEG derivatized streptavidin molecule was then determined using the displacement of 2-(4'-hydroxy-azobenzene)benzoic acid (HABA) from streptavidin by biotin. When HABA is bound to streptavidin there is a spectral shift at 500 nm which is proportional to the amount of bound HABA. HABA is quantitatively displaced from streptavidin by biotin. This enables the titration of the HABA-SA complex with biotin to be monitored directly by 500 nm absorbance, thereby providing a molar concentration of biotin binding sites. The ratio of binding site concentration to modified streptavidin concentration provides an indication of how the modification has disturbed biotin binding ability. (Unmodified streptavidin binds four biotin molecules.) The experimental protocol is as follows:

DEPR:

Subsequent experiments validated the utility of this method and the inventors' initial hypotheses. In vivo and in vitro experiments showed that streptavidin modified with PEG in the manner described herein reduced its immunogenicity to background levels. Also, the streptavidin derivatized with PEG in this manner was readily conjugated to antibody for antigen mediated targeting. Thus, these results demonstrate that polyethylene glycol derivatized ligands and anti-ligands, e.g., streptavidin and avidin, are useful in the preparation of conjugates for use in pretargeting methods. Such PEG derivatized ligands and anti-ligands are especially suitable for therapeutic pretargeting methods where immunogenicity may be a potential concern.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 11. Document ID: US 6210655 B1

L3: Entry 11 of 84

File: USPT

Apr 3, 2001

DOCUMENT-IDENTIFIER: US 6210655 B1
TITLE: Site-specific ¹³C-enriched reagents for diagnostic medicine by magnetic resonance imaging

BSPR:

Another example of imaging technology is the diagnosis of blood clots. Despite the frequency of pulmonary thromboembolism and its associated morbidity and mortality, diagnosis remains suboptimal. Similarly, noninvasive detection of both deep vein and cerebral thrombosis is currently difficult. Various radiolabeled proteins, such as antifibrin monoclonal antibodies, (Rosebrough, S. F. and Hashmi, M.: Galactose-modified streptavidin-GC4 antifibrin monoclonal antibody conjugates: application for two-step thrombus/embolus imaging. J. Pharm. Exp. Ther. 276(2): 770-775, 1996), fibrin-binding domain fragment of fibronectin (Rosenthal, L. and Leclerc, J.: A new thrombus imaging agent. Human recombinant fibrin binding domain labeled with In-111. Clin. Nucl. Med. 20(5): 398-402, 1995), activated-platelet binding protein fragment (Muto, P., Latoria, S., Varrella, P., et al.: Detecting deep venous thrombosis with technetium-^{99m}-labeled synthetic peptide P280. J. Nucl. Med. 36(8): 1384-1391, 1995) and (inactivated) tissue plasminogen activator (De Bruyn, V. H., Bergmann, S. R., Keyt, B. A. and Sobel, B. E.: Visualization of thrombi in pulmonary arteries with radiolabeled, enzymatically inactivated tissue-plasminogen activator. Circulation 92(5): 1320-1325, 1995) have been utilized for imaging thrombi.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 12. Document ID: US 6207390 B1

L3: Entry 12 of 84

File: USPT

Mar 27, 2001

DOCUMENT-IDENTIFIER: US 6207390 B1

TITLE: Methods for the use of reduced affinity streptavidin

ABPL:

The present invention relates to methods for contacting biological targets using a mutated streptavidin protein having a reduced affinity for biotin.

DRPR:

FIG. 1 Schematic of expression vector for a streptavidin mutant with a reduced binding affinity.

DEPR:

It has been discovered that a streptavidin mutant can be prepared with reduced affinity for biotin while still retaining a very high biotin binding specificity. Complexes formed with these mutants can be disrupted using only gentle treatment. This reduced-affinity streptavidin can be used in all situations which presently take advantage of the properties of wild-type streptavidin (SEQ ID NO:5). Previous difficulties associated with the presence of streptavidin or biotin in complexes are overcome.

DEPR:

The mutated streptavidin of Example 1 were used to produce large quantities Phe-120 streptavidin protein. Because the expression of streptavidin in bacteria has a lethal effect to a cell, an inducible system was used. The DNA fragment comprising the sequence encoding the streptavidin mutant was excised from its vector with the restriction endonucleases NdeI and BamHI, and cloned into the same sites in the T7 expression vector pET-3a. Resultant plasmids were transfected in BL21(DE3) (pLysE) bacteria.

DEPR:

Phe-120 streptavidin protein produced by induced E. coli was purified. Cells expressing the mutant streptavidin were harvested by centrifugation at 1600.times.g for 10 minutes. Protein was purified from the insoluble fraction of cell extracts. Briefly, cells were pelleted, washed with an isotonic solution of 100 mM NaCl, 1 mM EDTA and 10 mM Tris, pH 8.0, and resuspended in a detergent solution of 2 mM EDTA, 30 mM Tris-HCl, pH 8.0, 0.1% Triton X-100. Lysis occurred under these conditions because the presence of T7 lysozyme in the cells.

DEPR:

Impurities were removed by dialysis against 6 M guanidine hydrochloride pH 1.5. Mutant streptavidin was renatured slowly by dialysis against 0.2 M ammonium acetate, pH 6. After renaturation, insoluble impurities were removed by centrifugation at 39,000.times.g. Supernatant containing the mutant streptavidin protein was removed and collected. Final purifications were performed by 2-iminobiotin affinity chromatography.

DEPR:

On gel filtration chromatography using a Superdex 75 HR 10/30 column the molecular mass of purified Phe-120 was estimated to be 49,000 daltons, consistent with proper tetramer formation. Biotin bound Phe-120 was isolated also by Superdex 75 HR 10/30 column. When the biotin bound Phe-120 was analyzed, biotin was found at an amount of greater than 0.97 molecules of

biotin per streptavidin subunit. This degree of binding is consistent with full biotin-binding ability. These results indicate that Phe-120 streptavidin forms a tetramer as does wild type streptavidin (SEQ ID NO:5) and that the conversion of Trp-120 to Phe-120 has no significant effect on the basic properties of the mutant streptavidin. These data also indicate that the mutation had minimal effects on local environments around the biotin-binding sites and the dimer--dimer interface, thus allowing the correct folding of the molecule.

DEPR:

The biotin-binding affinities of wild type and Phe-120 streptavidin were determined by an equilibrium dialysis method using a micro dialyzer (Hoeffer Scientific). One hundred microliters each of D-[carbonyl-¹⁴C] biotin (2 nM-4 μ M; 53 mCi/mmol; Amersham) and 100 μ l of streptavidin (5.3 μ g/ml, 0.42 μ M subunits) were prepared separately in TBS (150 mM NaCl, 20 mM Tris-HCl, pH 7.4, 0.02% NaN₃) solutions. Equilibrium dialysis analysis was begun by the placement of the two solutions into two opposing chambers of a micro dialyzer. Chambers were incubated at 30.degree. C. with rotation for 48 hours and the concentration of labeled biotin in each chamber was measured by scintillation counting. Results were plotted on a Scatchard plot. The apparent biotin-binding affinity of the Phe-120 streptavidin was determined to be from about 1-3.times.10⁻⁸ M⁻¹ demonstrating the reduced substrate affinity of this streptavidin mutant.

DEPR:

Reduced substrate affinity streptavidin was dialyzed extensively against binding buffer (0.5 M sodium phosphate, pH 7.5) to remove inhibitors before attaching to solid supports. Cyanogen bromide activated beads (Pharmacia Biotech; Piscataway, N.J.) were washed with 100 volumes of binding buffer to remove preservatives and used immediately for coupling. Mutant streptavidin was crosslinked to the beads by adding the activated beads to the mutant streptavidin and mixing gently overnight at room temperature. Uncrosslinked mutant streptavidin was removed by washing the beads with binding buffer followed by a solution of 1 M NaCl and 0.05 M sodium phosphate, pH 7.5. Unreacted groups were blocked by incubating the beads in 100 mM ethanolamine, pH 7.5, for 4 hours with gentle mixing. After ethanolamine was removed by washes with phosphate buffered saline (PBS; 0.144 g/L KH₂PO₄, 9 g/L NaCl, 0.795 g/L Na₂HPO₄ -7H₂O), the reduced substrate affinity streptavidin coupled beads were ready for use.

DEPR:

An oncogene expression profile of a tumor can be determined by successive probing of a western blot using a variety of antibodies conjugated to ¹²⁵I-labeled streptavidin. Briefly, 500 μ Ci of ¹²⁵I-labeled N-succinimidyl 3-(4-hydroxyphenyl propionate) (ICN Radiochemicals; Irvine, Calif.) in dimethylformamide is air dried to the bottom of a tube. Radiolabeling is initiated by the addition of 10 μ g of reduced substrate annuity streptavidin in 10 μ l of 0.1 M sodium borate to the tube. Mutant streptavidin is labeled on ice for 15 minutes and the reaction terminated with 100 μ l of 0.5 M ethanolamine, 10% glycerol, 0.1% xylene cyanol and 0.1 M sodium borate, pH 8.5. Labeled streptavidin is separated from the labeling reagent on a gel filtration column.

DEPR:

An acidic residue is added to streptavidin to stabilize the dimeric and heterotetrameric forms of streptavidin. A single stranded DNA preparation of the resulting plasmid of Example 13, encoding core streptavidin with codons 113 to 120 deleted, is used as the starting material for this mutation. A single stranded DNA preparation is performed using E. coli as the host bacteria. An oligonucleotide, complementary to the single stranded DNA and encoding for aspartic acid at codon 127 of the streptavidin gene is synthesized with an oligonucleotide synthesizer and phosphorylated. Except for this mutagenic oligonucleotide, the procedure used for this mutagenesis is identical to oligonucleotide-directed deletion of residues 113 to 120. This procedure resulted in the production of a gene encoding core streptavidin with

a deletion of codon 113 to 120, and with aspartic acid at residue 127. The sequence of the resulting plasmid is confirmed by DNA sequencing using a dideoxy termination method. This gene is cloned into a bacterial expression vector and the mutated streptavidin expressed and purified. This streptavidin mutant dimerizes in aqueous solutions, has a reduced biotin binding affinity of less than 10×10^{-8} M, and can form heterodimers with the mutant streptavidin of Example 15.

DEPR:

A basic residue is added to streptavidin to stabilize the dimeric and heterotetrameric forms of streptavidin. A single stranded DNA preparation of the resulting plasmid of Example 13, encoding core streptavidin with codons 113 to 120 deleted, is used as the starting material. An oligonucleotide, complementary to the single stranded phage DNA and encoding for lysine at codon 127 of the streptavidin gene is synthesized with an oligonucleotide synthesizer and phosphorylated. Except for this mutagenic oligonucleotide, the procedure used for this mutagenesis is identical to oligonucleotide-directed deletion of residues 113 to 120. This procedure resulted in the production of a gene encoding core streptavidin with a deletion of codon 113 to 120, and with Aspartic acid at residue 127. The sequence of the resulting plasmid is confirmed by DNA sequencing using a dideoxy termination method. This gene is cloned into a bacterial expression vector and the mutated streptavidin is expressed and purified. This streptavidin mutant dimerizes in solution and forms a heterotetramer with the mutated streptavidin of Example 14. The biotin binding affinity of this mutant is less than 10×10^{-8} M.

DEPR:

Cysteine residues are added to the carboxyl terminus of streptavidin to allow conjugation to other molecules through sulfhydryl reactions. A plasmid DNA, encoding residues 16 to 112 and 121 to 133 of streptavidin with Lys at position 127 from example 15 is used as the starting material. Plasmid is digested with EcoRI and BamHI. Two 21 mer oligonucleotides, 5'-AAT TGC TGC TGC TGC TGC TAA-3' (SEQ ID NO 3), 5'-GAT CTT AGC AGC AGC AGC AGC AGC-3' (SEQ ID NO 4) are annealed, and the resulting double-stranded DNA inserted and ligated into the Eco RI and Bam HI sites of the predigested plasmid. The sequence of the resulting plasmid is confirmed by DNA sequencing using a dideoxy termination method. This gene is cloned into a bacterial expression vector and the mutated streptavidin expressed and purified. This streptavidin mutant has all the properties of the streptavidin mutant of Example 15. It dimerizes in solution, forms heterodimers with the streptavidin mutant of Example 14, and has a reduced biotin-binding affinity of less than about 10×10^{-8} M. In addition, this streptavidin mutant may be conjugated to other proteins and macromolecules, and also solid supports through the sulfhydryl group on the cysteines.

ORPL:

Sano et al., A Streptavidin Mutant Containing a Cysteine Stretch That Facilitates Production of a Variety of Specific Streptavidin Conjugates, Bio/Technology, Feb. 1993, vol. 11, pp. 201-206.*

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KMJC	Draw	Desc	Image
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☐ 13. Document ID: US 6204389 B1

L3: Entry 13 of 84

File: USPT

Mar 20, 2001

DOCUMENT-IDENTIFIER: US 6204389 B1

TITLE: Cyanine dyes and synthesis methods thereof

DEPR:

Streptavidin (15 mg) is dissolved in 1 mL 0.1M sodium phosphate buffer, pH 7.0. Sulfosuccinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (9 mg) in 0.2 mL dimethylsulfoxide is added to the streptavidin solution at room temperature, mixed for 1 hour and purified by gel filtration chromatography to give maleimide derivatized streptavidin. To a 1.5 mL solution of maleimide derivatized streptavidin (3.5 mg) in 0.1M sodium phosphate, 5 mM EDTA buffer, pH 6.0, 1 mg of Cyanine 3 Dye (15) is added followed by 0.15 mL of IM hydroxylamine pH 7.0. The solution is mixed at room temperature for 1 hour and then 20 .mu.L of 0.1M N-ethylmaleimide is added and the mixture is stored at 4.degree. C. for 16 hours. The mixture is purified by gel filtration chromatography to give streptavidin modified with Cyanine 3 Dye (15).

DEPR:

Streptavidin (15 mg) is dissolved in 1 mL 0.1M sodium phosphate buffer, pH 7.0. Sulfosuccinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (9 mg) in 0.2 mL dimethylsulfoxide is added to the streptavidin solution at room temperature, mixed for 1 hour and purified by gel filtration chromatography to give maleimide derivatized streptavidin. To a 1.5 .mu.L solution of maleimide derivatized streptavidin (3.5 mg) in 0.1M sodium phosphate, 5 mM EDTA buffer, pH 6.0, 1 mg of Cyanine 3 Dye (16) is added followed by 0.15 mL of IM hydroxylamine pH 7.0. The solution is mixed at room temperature for 1 hour and then 20 .mu.L of 0.1M N-ethylmaleimide is added and the mixture is stored at 4.degree. C. for 16 hours. The mixture is purified by gel filtration chromatography to give streptavidin modified with Cyanine 3 Dye (16).

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KMCC	Draw Desc	Image
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☐ 14. Document ID: US 6197956 B1

L3: Entry 14 of 84

File: USPT

Mar 6, 2001

DOCUMENT-IDENTIFIER: US 6197956 B1
TITLE: Cyanine dyes and synthesis methods thereof

DEPR:

Streptavidin (15 mg) is dissolved in 1 mL 0.1M sodium phosphate buffer, pH 7.0. Sulfosuccinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (9 mg) in 0.2 mL dimethylsulfoxide is added to the streptavidin solution at room temperature, mixed for 1 hour and purified by gel filtration chromatography to give maleimide derivatized streptavidin. To a 1.5 mL solution of maleimide derivatized streptavidin (3.5 mg) in 0.1M sodium phosphate, 5 mM EDTA buffer, pH 6.0, 1 mg of Cyanine 3 Dye (15) is added followed by 0.15 mL of 1M hydroxylamine pH 7.0. The solution is mixed at room temperature for 1 hour and then 20 .mu.L of 0.1M N-ethylmaleimide is added and the mixture is stored at 4.degree. C. for 16 hours. The mixture is purified by gel filtration chromatography to give streptavidin modified with Cyanine 3 Dye (15).

DEPR:

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Full	Title	Citation	Front	Review	Classification	Date	Reference
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RMIC	Draw Desc	Image
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☐ 15. Document ID: US 6165750 A

L3: Entry 15 of 84

File: USPT

Dec 26, 2000

DOCUMENT-IDENTIFIER: US 6165750 A
TITLE: Modified-affinity streptavidin

ABPL:

Streptavidin tetramers have at least one monomer containing an amino acid modification that produces a reduced binding affinity for biotin, a modified off-rate, a modified on-rate, or an altered binding enthalpy. Polynucleotides encoding the modified monomers are also provided. The modified streptavidin and chimeric streptavidin molecules are useful in methods of bioseparations and cell sorting, imaging, drug delivery, and diagnostics.

BSPR:

What is needed in the art is the ability to tailor the functional properties of individual subunits, and their geometrical distribution within the tetramer. This can be accomplished by manipulating important streptavidin structure-function relationships. A library of streptavidin mutants spanning a range of affinities and off- and on-rates for biotin and its derivatives would improve upon existing biotechnological applications for this already widely used system and open it to important new uses. Similarly, the ability to precisely define the subunit components and geometry will dramatically improve existing applications and provide new tools for cell separations, imaging, therapeutics and a variety of other technologies. Quite surprisingly, the

present invention fulfills these and other related needs.

BSPR:

In other aspects the invention provides a method for producing a streptavidin tetramer with at least one monomer thereof having a characteristic not found in a native streptavidin monomer subunit which characteristic affects affinity but not specificity for biotin. The method comprises producing altered streptavidin tetramer having the characteristic, separating the altered streptavidin into monomer and/or dimer subunits, e.g., by guanidium thiocyanate refolding; and mixing the streptavidin monomer and/or dimer subunits with streptavidin monomer or dimer subunits which do not have the characteristic, thereby producing a streptavidin tetramer having at least one monomer thereof with said characteristic. The method can also be used to assemble chimeric tetramers where at least one of the monomers but less than all contains a label, drug, toxin, targeting molecule, metal, or an amino acid modification that results in a reduced binding affinity of streptavidin for biotin. For example, the streptavidin monomer may contain at least one mutation in the amino acid sequence thereof which is situated at the dimer/dimer interface, e.g., a disulfide bond can be engineered to connect specific subunits and define the stoichiometry of the dissociated species.

DEPR:

In certain embodiments described herein the streptavidin subunits have a reduced binding affinity for biotin (or iminobiotin), e.g., by means of amino acid substitutions or deletions in residues of the biotin binding domain, and especially by changing the Trp residues at positions 79, 92, 108 and/or 120. Such characteristic resulting in the reduced binding affinity are accomplished by recombinant DNA techniques, where the specific amino acid residues of the streptavidin polypeptide are altered, e.g., by site-directed mutagenesis. Although examples of mutations which result in diminished affinity of the streptavidin polypeptide for biotin are described in the Example section below, where alterations at Trp79, Trp92, Trp108 and Trp120 result in a diminished binding, additional alterations based on the teachings hereof may be employed. For example, any of the amino acids other than Trp may be substituted at the selected position(s). The reduced binding affinities of the altered streptavidin for biotin (and/or iminobiotin) will typically be less than approximately $1 \times 10^{13} \text{ M}^{-1}$, sometimes less than about $1 \times 10^{12} \text{ M}^{-1}$, sometimes less than or equal to about $1 \times 10^{11} \text{ M}^{-1}$, and in some cases as low as about $1 \times 10^{10} \text{ M}^{-1}$ or lower, e.g., $1 \times 10^7 \text{ M}^{-1}$.

DEPR:

For example, methods for immunoselection of cells using avidin and biotin, in which the lower affinity mutants of the present invention are particularly useful, are described in detail in Berenson et al., U.S. Pat. No. 5,215,927, incorporated herein by reference. In one method for separating a target substance such as a hematopoietic stem cell from a heterogeneous suspension containing the target substance, a suspension such as a cell culture, bone marrow, peripheral blood or cord blood is reacted with a biotinylated binding component which binds to the target substance, thereby forming a biotinylated targeted substance complex. The biotinylated binding component can be an antibody, polyclonal, monoclonal or binding fragment thereof, that binds specifically to CD34+ hematopoietic stem cells. The suspension containing the biotinylated target substance complex is exposed to a modified streptavidin molecule of the present invention, e.g., a chimeric tetramer comprising at least one modified monomer but less than four modified monomers. For convenience, the exposing step can be performed in a column in which the streptavidin tetramer of the invention has been adsorbed to a solid phase. The biotinylated target substance complex is separated from the suspension by means of the lower affinity (or increase in dissociation rate constant) of the modified streptavidin monomer units to recover the target substance in enriched form.

DEPR:

Oligodeoxyribonucleotides, chemically 5'-phosphorylated during synthesis (IDT

Inc.), spanning the MluI/HindIII endonuclease restriction sites with degenerate codons at residue 120 incorporating the Trp->Ala/Phe mutation, were annealed, ligated into MluI/HindIII linearized pUC18 containing the core streptavidin gene, and transformed into competent Novablu cells. Clones containing mutant plasmid were identified by the insertion of a PstI restriction site upon successful ligation of the mutagenic insert into the core streptavidin gene. DNA sequencing of plasmids containing a PstI restriction site was used to identify clones containing either the Phe or Ala mutation. The mutant streptavidin genes (W120A or W120F) were subcloned into pET21a as an NdeI/HindIII segment, and maintained in Novablu cells.

DEPR:

Concentrations of the mutants were determined by the method of Gill et al., Anal. Biochem. 182: 319-326 (1989), using e.sub.280 of WT streptavidin as reference. Protein electrospray mass spectrometry (ESMS) was carried out on an API III electrospray mass spectrometer (PE/Sciex, Thornhill, Ontario). The biotin-binding stoichiometry of WT streptavidin and mutants was determined in solution by the quantitative quenching in the fluorescence of 5-((N-(5-(N-(6-(biotinyl)amino)hexanoyl) amino)pentyl) thioureydyl)fluorescein (Fluorescein-biotin, Molecular Probes, Eugene, OR) upon titration with protein.

DEPR:

A modified version of the enzyme assay reported by Bayer et al., Anal. Biochem. 154: 367-370 (1986), was used to examine the concentration-dependent binding of WT streptavidin and mutants with biotin and 2-iminobiotin. 2-Iminobiotin conjugated to bovine serum albumin (iminobiotin/BSA) was synthesized by reacting a twenty-fold molar excess of 2-iminobiotin N-hydroxysuccinimide ester (Sigma) with 100 mg BSA in 100 mM NaHCO.sub.3, pH 8.3 for 12 hr at 4.degree. C. with end-over-end stirring, followed by dialysis and gel filtration (G-25, Pharmacia) to separate iminobiotin/BSA from unreacted 2-iminobiotin. Biotin/BSA (Pierce) or iminobiotin/BSA at a concentration of 10 mg/ml were adsorbed overnight at 4.degree. C. in 15 mM Na.sub.2 CO.sub.3, pH 9.6, in microwell plates (100 .mu.l per well). The next day the microwell plates were incubated with 200 .mu.l per well of blocking buffer at pH 8.0 or pH 10.0 (50 mM sodium phosphate, pH 8.0 or 50 mM Na.sub.2 CO.sub.3, pH 10.0/100 mM NaCl/0.5% (w/v) BSA/0.05% (v/v) Tween-20) for at least 2 hr at room temperature and then incubated in ten-fold serial dilutions of 100 .mu.g/ml WT streptavidin or mutants (100 .mu.l/well) for 2 hr at room temperature, rinsed with blocking buffer at pH 8.0 or pH 10.0 (200 .mu.l/well), and incubated for 1 hr at room temperature in 2.times.10.sup.4 dilution of primary antistreptavidin antibodies (Sigma) in pH 8.0 or pH 10.0 blocking buffer (100 .mu.l/well). The plates were rinsed three times with 200 .mu.l per well blocking buffer at the assay pH, incubated with secondary anti-IgG/alkaline phosphatase conjugate (Sigma) for 1 hr at room temperature, rinsed three times with blocking buffer, and assayed for alkaline phosphatase activity at pH 10.0. Every plate assayed had triplicates for each protein concentration. The data were processed on Mathcad (Mathworks) to determine the equivalent bulk concentration at 50% saturation of binding (EC.sub.50) values using a published 4-parameter nonlinear fitting algorithm shown below (Jin et al., J. Mol. Biol. 226: 851-865 (1992)):

DEPR:

With biotin as the ligand, the concentration-dependent binding isotherms of WT streptavidin and all Trp mutants are largely identical in the ELISA assay at both pH 8.0 and 10 (FIG. 2A shows the binding isotherm of WT streptavidin and the W79A/F mutants). However, with iminobiotin/BSA as the ligand (FIG. 2B for WT streptavidin and the W79A/F mutants) the binding isotherms indicate marked differences in the affinities of WT streptavidin and the mutants, with the EC.sub.50's in the order WT

DEPR:

The equilibrium binding enthalpies, .DELTA.H.degree., and the heat capacities, .DELTA.Cp.degree., have also been engineered with the streptavidin mutants (Table II). Heat capacities were determined as generally described in Murphy et al., Proteins: Structure, Function and Genetics 15:113-120 (1993),

incorporated herein by reference. As can be seen, there are examples of both increased and decreased .DELTA.H.degree. and increased and decreased .DELTA.Cp.degree.. The heat capacities relate the temperature dependence of the binding enthalpy, which contributes significantly to the binding affinity. Alterations in the heat capacity are thus important in applications where temperature is used as a variable to control the biotin affinity.

DEPR:

This Example demonstrates the contribution of binding-site tryptophan residues to the biotin off rate and activation thermodynamics. The dissociation rate constants of streptavidin mutants W79F, W108F and W120F indicate these Trp contacts are important in regulating the dissociation rate.

DEPL:

Expression of Streptavidin and Mutants in E. coli

DEPL:

Isolation and Purification of Expressed Streptavidin and Mutants

DEPL:

Characterization of Streptavidin and Mutants

Full	Title	Citation	Front	Review	Classification	Date	Reference
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NAME	Draw Desc	Image
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☐ 16. Document ID: US 6156493 A

L3: Entry 16 of 84

File: USPT

Dec 5, 2000

DOCUMENT-IDENTIFIER: US 6156493 A

TITLE: Modified-affinity streptavidin

ABPL:

Streptavidin tetramers have at least one monomer containing an amino acid modification that produces a reduced binding affinity for biotin, a modified off-rate, a modified on-rate, or an altered binding enthalpy. Polynucleotides encoding the modified monomers are also provided. The modified streptavidin and chimeric streptavidin molecules are useful in methods of bioseparations and cell sorting, imaging, drug delivery, and diagnostics.

BSPR:

What is needed in the art is the ability to tailor the functional properties of individual subunits, and their geometrical distribution within the tetramer. This can be accomplished by manipulating important streptavidin structure-function relationships. A library of streptavidin mutants spanning a range of affinities and off- and on-rates for biotin and its derivatives would improve upon existing biotechnological applications for this already widely used system and open it to important new uses. Similarly, the ability to precisely define the subunit components and geometry will dramatically improve existing applications and provide new tools for cell separations, imaging, therapeutics and a variety of other technologies. Quite surprisingly, the present invention fulfills these and other related needs.

BSPR:

In other aspects the invention provides a method for producing a streptavidin tetramer with at least one monomer thereof having a characteristic not found in a native streptavidin monomer subunit which characteristic affects affinity but not specificity for biotin. The method comprises producing altered streptavidin tetramer having the characteristic, separating the altered streptavidin into monomer and/or dimer subunits, e.g., by guanidium

thiocyanate refolding; and mixing the streptavidin monomer and/or dimer subunits with streptavidin monomer or dimer subunits which do not have the characteristic, thereby producing a streptavidin tetramer having at least one monomer thereof with said characteristic. The method can also be used to assemble chimeric tetramers where at least one of the monomers but less than all contains a label, drug, toxin, targeting molecule, metal, or an amino acid modification that results in a reduced binding affinity of streptavidin for biotin. For example, the streptavidin monomer may contain at least one mutation in the amino acid sequence thereof which is situated at the dimer/dimer interface, e.g., a disulfide bond can be engineered to connect specific subunits and define the stoichiometry of the dissociated species.

DEPR:

In certain embodiments described herein the streptavidin subunits have a reduced binding affinity for biotin (or iminobiotin), e.g., by means of amino acid substitutions or deletions in residues of the biotin binding domain, and especially by changing the Trp residues at positions 79, 92, 108 and/or 120. Such characteristic resulting in the reduced binding affinity are accomplished by recombinant DNA techniques, where the specific amino acid residues of the streptavidin polypeptide are altered, e.g., by site-directed mutagenesis. Although examples of mutations which result in diminished affinity of the streptavidin polypeptide for biotin are described in the Example section below, where alterations at Trp79, Trp92, Trp108 and Trp120 result in a diminished binding, additional alterations based on the teachings hereof may be employed. For example, any of the amino acids other than Trp may be substituted at the selected position(s). The reduced binding affinities of the altered streptavidin for biotin (and/or iminobiotin) will typically be less than approximately 1.times.10.sup.13 M.sup.-1, sometimes less than about 1.times.10.sup.12 M.sup.-1, sometimes less than or equal to about 1.times.10.sup.11 M.sup.-1, and in some cases as low as about 1.times.10.sup.10 M.sup.-1 or lower, e.g., 1.times.10.sup.7 M.sup.-1.

DEPR:

For example, methods for immunoselection of cells using avidin and biotin, in which the lower affinity mutants of the present invention are particularly useful, are described in detail in Berenson et al., U.S. Pat. No. 5,215,927, incorporated herein by reference. In one method for separating a target substance such as a hematopoietic stem cell from a heterogeneous suspension containing the target substance, a suspension such as a cell culture, bone marrow, peripheral blood or cord blood is reacted with a biotinylated binding component which binds to the target substance, thereby forming a biotinylated targeted substance complex. The biotinylated binding component can be an antibody, polyclonal, monoclonal or binding fragment thereof, that binds specifically to CD34+ hematopoietic stem cells. The suspension containing the biotinylated target substance complex is exposed to a modified streptavidin molecule of the present invention, e.g., a chimeric tetramer comprising at least one modified monomer but less than four modified monomers. For convenience, the exposing step can be performed in a column in which the streptavidin tetramer of the invention has been adsorbed to a solid phase. The biotinylated target substance complex is separated from the suspension by means of the lower affinity (or increase in dissociation rate constant) of the modified streptavidin monomer units to recover the target substance in enriched form.

DEPR:

Oligodeoxyribonucleotides, chemically 5'-phosphorylated during synthesis (IDT Inc.), spanning the MluI/HindIII endonuclease restriction sites with degenerate codons at residue 120 incorporating the Trp.fwdarw.Ala/Phe mutation, were annealed, ligated into MluI/HindIII linearized pUC18 containing the core streptavidin gene, and transformed into competent Novablu cells. Clones containing mutant plasmid were identified by the insertion of a PstI restriction site upon successful ligation of the mutagenic insert into the core streptavidin gene. DNA sequencing of plasmids containing a PstI restriction site was used to identify clones containing either the Phe or Ala mutation. The mutant streptavidin genes (W120A or W120F) were subcloned into

pET21a as an NdeI/HindIII segment, and maintained in Novablue cells.

DEPR:

Expression of Streptavidin and Mutants in E. coli. The expression plasmid pET-21a containing the streptavidin gene, transformed into the expression host, BL21(DE3) (Novagen, Inc.), was cultured overnight at 37.degree. C. with shaking, in 10 ml LB medium supplemented with 100 .mu.g/ml ampicillin. The culture was then centrifuged at 4500.times.g for 5 min, the cell pellet resuspended in fresh 10 ml LB medium and used to inoculate 6.5 L of 2.times. YT medium supplemented with 100 .mu.g/ml ampicillin in shaker flasks. The culture was incubated with shaking at 37.degree. C. until the absorbance at 600 nm reached 1.0, at which point isopropyl .beta.-D-thiogalactopyranoside was added (1 mM) to induce protein expression. Cells were cultured for a further 3 hrs, after which they were harvested by centrifugation at 4500.times.g for 10 min. The cell pellets were stored at -70.degree. C. until further use. Quantitation of protein bands in SDS-polyacrylamide gel electrophoresis (PAGE) of induced BL21(DE3) cell lysates by laser densitometry revealed that streptavidin constituted 15-20% of the total cellular protein.

DEPR:

Isolation and Purification of Expressed streptavidin and Mutants. The frozen cell pellet was thawed, resuspended in 200 ml 50 mM Tris HCl, pH 8.0/0.75 M sucrose/1 mM phenylmethylsulfonyl fluoride (PMSF), and ruptured by sonication. The lysed cells were incubated at room temperature for 15 min with DNaseI (10 .mu.g/ml)/RNaseA (10 .mu.g/ml)/MgCl.sub.2 (10 mM), and centrifuged at 22000.times.g for 30 min. The insoluble fraction was repeatedly washed with 50 mM Tris, pH 8.0/10 mM EDTA/1.5 M NaCl/1 mM PMSF/0.5% (v/v) Triton-X-100 to solubilize membrane proteins. The final white pellet, largely comprising streptavidin inclusion bodies, was approximately 70% pure, as determined by SDS-PAGE. The inclusion bodies were dissolved in 6M guanidine HCl (500 ml), pH 1.5, to a concentration.ltoreq.50 .mu.M (streptavidin monomer) and dialyzed against 20 liters 50 mM Tris HCl, pH 8.0/150 mM NaCl/10 mM EDTA/0.1 mM PMSF/0.5 mM benzamidinium HCl over 24 h with one 20 L change of dialysis buffer. The dialysate was centrifuged, vacuum filtered through 0.45 .mu.M filters, and concentrated in a stirred ultrafiltration cell (Amicon Inc., Danvers, Mass.). Final concentration to a few ml utilized Centriprep-30 centrifugal concentrators (Amicon Inc.). Insoluble, aggregated protein left over after dialysis can be refolded several times by following the above protocol.

DEPR:

Characterization of Streptavidin and Mutants. SDS-PAGE analysis was carried out using precast Miniprotean 10-20% gradient gels (BioRad Inc., Richmond, Calif.) with a discontinuous buffer system (Laemmli, Nature 227: 680-685 (1971)). Samples were boiled for 15 min in the presence of SDS before electrophoresis to dissociate streptavidin tetramers. Native-PAGE was performed using the above system by omitting SDS in the sample application buffer and the gel running buffer, as well as the heat denaturation of proteins before electrophoresis. The gels were stained with 0.25% (w/v) Coomassie R-250, dissolved in 45% methanol (v/v), 10% acetic acid (v/v). The concentration of WT streptavidin was determined by absorbance at 280 nm using an extinction coefficient (e.sub.280) of 34000 M.sup.-1 cm.sup.-1 for the subunit (Sano et al., Proc. Natl. Acad. Sci. USA 87: 142-146 (1990)). Concentrations of the mutants were determined by the method of Gill et al., Anal. Biochem. 182: 319-326 (1989), using e.sub.280 of WT streptavidin as reference. Protein electrospray mass spectrometry (ESMS) was carried out on an API III electrospray mass spectrometer (PE/Sciex, Thornhill, Ontario). The biotin-binding stoichiometry of WT streptavidin and mutants was determined in solution by the quantitative quenching in the fluorescence of 5-((N-(5-(N-(6-(biotinyl)amino)hexanoyl)amino)pentyl)thioureidyl)fluorescein (Fluorescein-biotin, Molecular Probes, Eugene, Oreg.) upon titration with protein.

DEPR:

ELISA Assays. A modified version of the enzyme assay reported by Bayer et al., Anal. Biochem. 154: 367-370 (1986), was used to examine the

concentration-dependent binding of WT streptavidin and mutants with biotin and 2-iminobiotin. 2-Iminobiotin conjugated to bovine serum albumin (iminobiotin/BSA) was synthesized by reacting a twenty-fold molar excess of 2-iminobiotin N-hydroxysuccinimide ester (sigma) with 100 mg BSA in 100 mM NaHCO₃, pH 8.3 for 12 hr at 4.degree. C. with end-over-end stirring, followed by dialysis and gel filtration (G-25, Pharmacia) to separate iminobiotin/BSA from unreacted 2-iminobiotin. Biotin/BSA (Pierce) or iminobiotin/BSA at a concentration of 10 mg/ml were adsorbed overnight at 4.degree. C. in 15 mM Na₂CO₃, pH 9.6, in microwell plates (100 .mu.l per well). The next day the microwell plates were incubated with 200 .mu.l per well of blocking buffer at pH 8.0 or pH 10.0 (50 mM sodium phosphate, pH 8.0 or 50 mM Na₂CO₃, pH 10.0/100 mM NaCl/0.5% (w/v) BSA/0.05% (v/v) Tween-20) for at least 2 hr at room temperature and then incubated in ten-fold serial dilutions of 100 .mu.g/ml WT streptavidin or mutants (100 .mu.l/well) for 2 hr at room temperature, rinsed with blocking buffer at pH 8.0 or pH 10.0 (200 .mu.l/well), and incubated for 1 hr at room temperature in 2.times.10.sup.4 dilution of primary antistreptavidin antibodies (Sigma) in pH 8.0 or pH 10.0 blocking buffer (100 .mu.l/well). The plates were rinsed three times with 200 .mu.l per well blocking buffer at the assay pH, incubated with secondary anti-IgG/alkaline phosphatase conjugate (Sigma) for 1 hr at room temperature, rinsed three times with blocking buffer, and assayed for alkaline phosphatase activity at pH 10.0. Every plate assayed had triplicates for each protein concentration. The data were processed on Mathcad (Mathworks) to determine the equivalent bulk concentration at 50% saturation of binding (EC₅₀) values using a published 4-parameter nonlinear fitting algorithm shown below (Jin et al., J. Mol. Biol. 226: 851-865 (1992)):

DEPR:

With biotin as the ligand, the concentration-dependent binding isotherms of WT streptavidin and all Trp mutants are largely identical in the ELISA assay at both pH 8.0 and 10 (FIG. 2A shows the binding isotherm of WT streptavidin and the W79A/F mutants). However, with iminobiotin/BSA as the ligand (FIG. 2B for WT streptavidin and the W79A/F mutants) the binding isotherms indicate marked differences in the affinities of WT streptavidin and the mutants, with the EC₅₀'s in the order WT

DEPR:

The equilibrium binding enthalpies, .DELTA.H.degree., and the heat capacities, .DELTA.Cp.degree., have also been engineered with the streptavidin mutants (Table II). Heat capacities were determined as generally described in Murphy et al., Proteins: Structure, Function and Genetics 15:113-120 (1993), incorporated herein by reference. As can be seen, there are examples of both increased and decreased .DELTA.H.degree. and increased and decreased .DELTA.Cp.degree.. The heat capacities relate the temperature dependence of the binding enthalpy, which contributes significantly to the binding affinity. Alterations in the heat capacity are thus important in applications where temperature is used as a variable to control the biotin affinity.

DEPR:

This Example demonstrates the contribution of binding-site tryptophan residues to the biotin off rate and activation thermodynamics. The dissociation rate constants of streptavidin mutants W79F, W108F and W120F indicate these Trp contacts are important in regulating the dissociation rate.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 17. Document ID: US 6150503 A

L3: Entry 17 of 84

File: USPT

Nov 21, 2000

DOCUMENT-IDENTIFIER: US 6150503 A
TITLE: Phosphorylated fusion proteins

BSPR:

The invention also provides particularly interesting labellable and labelled proteins like phosphorylated antibodies (especially monoclonal antibodies, hybrid antibodies, chimeric antibodies or modified antibodies), hormones, and "modified" streptavidin. The modified streptavidin can be bound to individual biotinylated antibodies, each streptavidin being modified by single or multiple phosphorylated groups, which product has greatly enhanced radiation and therefore diagnostic and therapeutic potential.

DEPR:

A particularly noteworthy and interesting application made possible by the invention is what has been called here in the vernacular, a therapeutic or more specifically an antitumor "therapeutic radiation bomb". Such a biologically-active composition uses biotin coupled to a tumor-specific monoclonal antibody (Mab) (or to Fab or Fab' fragments if more appropriate), and a multiple "modified" streptavidin bound to each Mab-bound biotin, each streptavidin being modified in that it has multiple phosphorylated groups. Since streptavidin is itself a tetramer, multiple radioactive groups are thus provided. These multiple radioactive groups expose the tumor with radiation which is greatly amplified and hence more readily detectable and would produce greater tumor destruction. In the case where it is highly phosphorylatable it is much more easily detectable. Thus, each one of the biotins which is bound to each tumor-specific Mab binds tightly to the multiple streptavidin molecules which in turn contain multiple labelled phosphorus atoms, or their equivalent isotopes.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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RWMC	Draw Desc	Image
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☐ 18. Document ID: US 6150089 A

L3: Entry 18 of 84

File: USPT

Nov 21, 2000

DOCUMENT-IDENTIFIER: US 6150089 A
TITLE: Method and characterizing polymer molecules or the like

DEPR:

Attached Fluorescent Beads for Optical recA Mediated hybridization Detection. Single fluorescent beads are easily imaged with fluorescence microscopy, including the smallest ones with a diameter of just 0.01 microns. (Although exceeding the Rayleigh limit, this bead appears as a bright spot.) Fluorescent beads are a good way to label single DNA molecules for image processing because individual beads are intensely fluorescent, morphologically distinctive, available in wide range of fluorochromes of differing spectral qualities, and are easily attached to oligonucleotides. For example, Molecular Probes, Inc., sells latex beads with coatings of carboxylate, avidin or streptavidin in 6 spectral ranges (colors) and sizes varying from 0.01 to 2 microns. The availability of carboxylate modified and streptavidin coated beads offers many alternatives for binding them to DNA molecules.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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RWMC	Draw Desc	Image
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☐ 19. Document ID: US 6147198 A

L3: Entry 19 of 84

File: USPT

Nov 14, 2000

DOCUMENT-IDENTIFIER: US 6147198 A

TITLE: Methods and compositions for the manipulation and characterization of individual nucleic acid molecules

DEPR:

In another embodiment, detection is based on fluorescent beads and on chemiluminescent tagging using alkaline phosphatase. Single fluorescent beads are easily imaged with fluorescence microscopy, including the smallest ones with a diameter of just 0.01 microns. (Although exceeding the Rayleigh limit, this bead appears as a bright spot.) Fluorescent beads provide a good way to label single DNA molecules for image processing purposes because individual beads are intensely fluorescent, morphologically distinctive, available in wide range of fluorochromes of differing spectral qualities, and are easily attached to oligonucleotides. For example, Molecular Probes, Inc., sells latex beads with coatings of carboxylate, avidin or streptavidin in 6 spectral ranges (colors) and sizes varying from 0.01 to 2 microns. The availability of carboxylate modified and streptavidin coated beads offers many alternatives for binding them to DNA molecules.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 20. Document ID: US 6127170 A

L3: Entry 20 of 84

File: USPT

Oct 3, 2000

DOCUMENT-IDENTIFIER: US 6127170 A

TITLE: Multifunctional complexes for gene transfer into cells comprising a nucleic acid bound to a polyamine and having an endosome disruption agent

BSPR:

Macrophages have receptors for both mannose and mannose-6-phosphate which can bind to and internalize molecules displaying these sugars. The molecules are internalized by endocytosis into a pre-lysosomal endosome. This internalization has been used to enhance entry of oligonucleotides into macrophages using bovine serum albumin modified with mannose-6-phosphate and linked to an oligodeoxynucleotide by a disulfide bridge to a modified 3' end; see E. Bonfils, C. Depierreux, P. Midoux, N. T. Thuong, M. Monsigny and A. C. Roche, Nucl. Acids Res. 20, 4621-4629 (1992). Similarly, oligodeoxynucleotides modified at the 3' end with biotin were combined with mannose-modified streptavidin, and were also found to have facilitated entry into macrophages; see E. Bonfils, C. Mendes, A. C. Roche, M. Monsigny and P. Midoux, Bioconj. Chem., 3, 277-284 (1992).

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 21. Document ID: US 6114350 A

L3: Entry 21 of 84

File: USPT

Sep 5, 2000

DOCUMENT-IDENTIFIER: US 6114350 A

TITLE: Cyanine dyes and synthesis methods thereof

DEPR:

Streptavidin (15 mg) is dissolved in 1 mL 0.1M sodium phosphate buffer, pH 7.0. Sulfosuccinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (9 mg) in 0.2 mL dimethylsulfoxide is added to the streptavidin solution at room temperature, mixed for 1 hour and purified by gel filtration chromatography to give maleimide derivatized streptavidin. To a 1.5 mL solution of maleimide derivatized streptavidin (3.5 mg) in 0.1M sodium phosphate, 5 mM EDTA buffer, pH 6.0, 1 mg of Cyanine 3 Dye (15) is added followed by 0.15 mL of 1M hydroxylamine pH 7.0. The solution is mixed at room temperature for 1 hour and then 20 .mu.L of 0.1M N-ethylmaleimide is added and the mixture is stored at 4.degree. C. for 16 hours. The mixture is purified by gel filtration chromatography to give streptavidin modified with Cyanine 3 Dye (15).

DEPR:

Streptavidin (15 mg) is dissolved in 1 mL 0.1M sodium phosphate buffer, pH 7.0. Sulfosuccinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (9 mg) in 0.2 mL dimethylsulfoxide is added to the streptavidin solution at room temperature, mixed for 1 hour and purified by gel filtration chromatography to give maleimide derivatized streptavidin. To a 1.5 mL solution of maleimide derivatized streptavidin (3.5 mg) in 0.1M sodium phosphate, 5 mM EDTA buffer, pH 6.0, 1 mg of Cyanine 3 Dye (16) is added followed by 0.15 mL of 1M hydroxylamine pH 7.0. The solution is mixed at room temperature for 1 hour and then 20 .mu.L of 0.1M N-ethylmaleimide is added and the mixture is stored at 4.degree. C. for 16 hours. The mixture is purified by gel filtration chromatography to give streptavidin modified with Cyanine 3 Dye (16).

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 22. Document ID: US 6103493 A

L3: Entry 22 of 84

File: USPT

Aug 15, 2000

DOCUMENT-IDENTIFIER: US 6103493 A

TITLE: Streptavidin muteins

DEPR:

The hybrid structural gene containing the coding sequences for the signal peptide and minimal streptavidin begins at the XbaI site and extends downstream to the HindIII site. The junction between the signal sequence and streptavidin is at the StuI/PvuII site. The SacII site which was used to insert the mutated streptavidin gene sequences is also shown.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 23. Document ID: US 6096508 A

L3: Entry 23 of 84

File: USPT

Aug 1, 2000

DOCUMENT-IDENTIFIER: US 6096508 A

TITLE: Method of reducing background in biotin-based assays

DEPR:

The wash step typically follows one or more of the steps of (i) sample immobilization on a solid phase, (ii) blocking the solid phase, as needed, to prevent non-specific binding of detector molecule or probe, (iii) contacting the sample with detector molecule or probe, (iv) washing the solid phase, including the sample, to remove excess (including non-specifically bound) detector molecule or probe, (v) blocking the solid phase to prevent non-specific binding of signaling moiety, and (vi) contacting the sample with signaling moiety to identify the presence of any detector molecule or probe bound to a target material in the sample. The present invention is particularly based on the surprising discovery that by including a background signal-reducing amount of free biotin in a standard wash solution in an assay based on the biotin and avidin or biotin and streptavidin interaction, i.e., an assay which uses biotin as the heterologous moiety and a modified avidin (e.g., an avidin-enzyme conjugate) or a modified streptavidin (e.g., a streptavidin-enzyme conjugate) as the signaling moiety, one can greatly reduce non-specific binding of the signaling moiety to sites other than the desired target. Thus, one can reduce the level of undesired background signal, with minimal effect on the specific signal resulting from desired binding of the signaling moiety to those biotin-labeled detector molecules or probes which are bound to the target. In those cases where the signaling moiety includes an enzyme, the wash step then is followed by contacting the sample with an indicator substrate for the enzyme.

DEPR:

Though not wishing to be limited to any particular technical explanation, applicants believe that free heterologous moiety, e.g., free biotin, in the wash solution preferentially binds to non-specifically bound signaling moiety, e.g., modified-avidin or modified-streptavidin, such as enzyme conjugates of avidin and streptavidin (i.e., signaling moiety not bound to the desired target), and preferentially enhances the removal of non-specifically bound signaling moiety prior to the detection and/or identification step.

DEPR:

The binding of avidin and streptavidin to biotin is one of the most avid bonds in nature. The strength of this bond gives detection systems based on the biotin/avidin and biotin/streptavidin interaction many advantages. Researchers in the biomedical field have long utilized modified streptavidin as detection intermediates (signaling moieties) in solid phase assays wherein a specific probe has been modified with biotin. In these assays, biotin has been chemically or enzymatically coupled to probe biomolecules in ways to minimize interference with target recognition. The most common application involves biotinylated nucleic acid probes used in solid phase assays wherein the target DNA or RNA samples are immobilized, for example, on a solid support such as nitrocellulose or nylon membranes. The specific probes are placed in contact with the sample, potentially containing the target sequence(s), and are allowed to hybridize. In some cases, the solid support containing the sample may first be treated with a heterologous, denatured DNA or with a protein, such as milk protein, to block non-specific hybridization of labeled probes. See Maniatis et al., Molecular Cloning: A Laboratory Manual, 2nd Edition, (1988), Cold Spring

DEPR:

The invention is specifically applicable to biotin-based detection systems using a wide variety of streptavidin-modified and avidin-modified signaling moieties, including streptavidin-enzyme conjugates or avidin-enzyme conjugates in addition to horseradish peroxidase conjugates. Techniques for preparing such modified streptavidin and modified avidin are known to those skilled in the art. Suitable enzymes for making conjugates with avidin and streptavidin include (i) horseradish peroxidase for which suitable chromogenic substrates include 3,3'-diaminobenzidine; 4-chloro-1-naphthol; 3-amino-9-ethylcarbazole and 3,3',5,5'-tetramethylbenzidine, suitable fluorogenic substrate include homovanillic acid and 4-hydroxy-3-methoxyphenylacetic acid, and a suitable

chemiluminescent substrate includes enhanced luminol, (ii) alkaline phosphatase for which a suitable chromogenic substrate is 5-bromo-4-chloro-3-indolyl-phosphate/nitroblue tetrazolium, a suitable fluorogenic substrate is 4-methylumbelliferyl phosphate or other umbelliferyl phosphates such as 4-carboxy-umbelliferyl phosphate and its alkyl esters and a suitable chemiluminescent substrate is 1,2-dioxetane, and (iii) beta-galactosidase for which a suitable chromogenic substrate is 5-bromo-4-chloro-3-indolyl-D-galactosidase, and a fluorogenic substrate is 4-methylumbelliferyl-beta-D-galactoside. Techniques for preparing such enzyme-labeled signaling moieties and particularly streptavidin- and avidin-enzyme conjugates is well understood by those skilled in the art.

DEPR:

In addition to enzyme-conjugated signaling moieties, which represent an indirect method for recognizing the presence of a detector molecule, the present invention also is directed to the use of signaling moieties that provide a direct signal for detection and/or identification. In this regard, the following signaling moieties can be mentioned, streptavidin or avidin modified with a fluorophore such as fluorescein, phycoerythrin or tetramethyl rhodamine, (useful in immunohistological and in situ hybridization applications), streptavidin modified with a colloidal moiety such as colloidal gold or a carbon sol (designed to provide a directly recognizable visual signal) and streptavidin modified with a radioisotope such as ¹²⁵I. As used herein, the terms "modified avidin" and "modified streptavidin" are intended to include modifications which produce a detection signal by both direct and indirect methods.

DEPR:

Although not wishing to be bound to any particular technical explanation, the success of the invention is apparently due to a favorable balance of competitive binding of the non-specifically bound signaling moiety, such as a modified streptavidin or modified avidin, by the free heterologous moiety, such as free biotin, in the wash solution. The nature of the free heterologous moiety (e.g., biotin) interaction between signaling moiety (e.g. modified streptavidin) bound to detector molecules and non-specifically bound signaling moiety could not have been predicted. An important aspect of the invention, however, is the discovery that any system which utilizes streptavidin or avidin binding to biotin, or an interaction similar to that between avidin and biotin, as the mechanism for detection can benefit from this novel method of reducing background level by employing as a wash solution, designed to remove non-specifically bound signaling moiety (e.g., modified streptavidin), a composition containing free heterologous moiety (e.g., free biotin).

CLPR:

1. In a method of detecting a target material in a sample immobilized on a solid phase support using a detector molecule, wherein the detector molecule binds to the target material, wherein said detector molecule has a heterologous moiety attached thereto, wherein said heterologous moiety attached to the detector molecule is detected by a signaling moiety, wherein the signaling moiety binds to the heterologous moiety, and wherein said method comprises a step of washing excess non-specifically bound signaling moiety with a wash solution from said solid phase support, the improvement comprising employing as said wash solution a composition comprising free heterologous moiety, wherein the heterologous moiety attached to the detector molecule is selected from the group consisting of biotin, biotin derivatives and biotin analogs; wherein the free heterologous moiety is selected from the group consisting of biotin, biotin derivatives and biotin analogs and wherein the signaling moiety is selected from the group consisting of modified streptavidin and modified avidin.

CLPR:

2. The method of claim 1 wherein the modified streptavidin is selected from the group consisting of streptavidin conjugated with an enzyme,

CLPR:

streptavidin modified with a fluorophore, streptavidin modified with a colloidal moiety and streptavidin modified with a radioisotope.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KMOC	Draw Desc	Image
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☐ 24. Document ID: US 6096500 A

L3: Entry 24 of 84

File: USPT

Aug 1, 2000

DOCUMENT-IDENTIFIER: US 6096500 A

TITLE: Multi-label complex for enhanced sensitivity in electrochemiluminescence assay

DEPR:

The beads also include a coating or other means for affixing the oligonucleotide capture probe to the bead. For example, the bead may be coated with streptavidin so as to bind and carry the oligonucleotide probe, the latter being biotinylated for reaction with the streptavidin. Alternatively, the bead may be biotinylated and the probe modified with streptavidin for attachment to the bead. Other alternatives or modifications are contemplated provided the result comprises a magnetic bead carrying an oligonucleotide probe for ECL use.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KMOC	Draw Desc	Image
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☐ 25. Document ID: US 6077663 A

L3: Entry 25 of 84

File: USPT

Jun 20, 2000

DOCUMENT-IDENTIFIER: US 6077663 A

TITLE: Composition for introducing nucleic acid complexes into higher eucaryotic cells

BSPR:

The invention also relates to a transfection kit, comprising a carrier means having in close confinement therein one or more container means, wherein a first container means contains a biotin-modified endosomolytic agent and a second container means contains a streptavidin-modified substance having affinity for nucleic acid.

DEPR:

The coupling of streptavidin to polylysine was effected using the method described by Wagner et al., 1990, and in EP-A1 388 758. 79 nmol (4.7 mg) of streptavidin in 1 ml of 200 mM HEPES pH 7.9 and 300 mM NaCl were treated with a 15 mM ethanolic solution of SPDP (236 nmol). After 1.5 hours at ambient temperature the modified protein was gel filtered over a Sephadex G-25 column, thereby obtaining 75 nmol of streptavidin, modified with 196 nmol of dithiopyridine linker. The modified protein was reacted under an argon atmosphere with 3-mercaptopropionate-modified polylysine (75 nmol, average chain length 290 lysine monomers, modified with 190 nmol mercapto-propionate linker) in 2.6 ml of 100 mM HEPES pH 7.9, 150 mM NaCl. Conjugates were isolated by cation exchange chromatography on a Mono S HR5 column (Pharmacia). (Gradient: 20-100% buffer B. Buffer A: 50 mM HEPES pH 7.9; buffer B: buffer A plus 3M sodium chloride). The product fraction eluted at a salt concentration

of between 1.2M and 1.7M. Dialysis against HBS (20 mM HEPES pH 7.3, 150 mM NaCl) resulted in a conjugate consisting of 45 nmol of streptavidin and 53 nmol of polylysine.

DEPR:

For formation of adenovirus-DNA-transferrin complexes 50 .mu.l of biotinylated adenovirus were incubated with 400 ng of streptavidin-modified polylysine in 20 .mu.l HBS for 20 min. Then 20 .mu.l of HBS containing 6 .mu.g pCMVL were added. After an incubation period for 20 min 7 .mu.g of mouse transferrin-polylysine conjugate (mTfpL) in 160 .mu.l HBS were added and the whole mixture was incubated for further 20 min.

DEPR:

The complexes were prepared as follows: 200 .mu.l biotinylated Adenovirus dl312 diluted with 200 .mu.l HBS were incubated with 6.4 .mu.g streptavidin-modified polylysine in 400 .mu.l HBS for 30 minutes at room temperature. Then 48 .mu.g of pCMV-L in 800 .mu.l HBS were added. After 30 minutes of incubation 48 .mu.g of TfpL in 900 .mu.l HBS were further added. For application of the complexes male Sprague Dawley rats (250 g body weight) were anesthetized with Avertin and the abdomen opened with a median incision. The intestine was displaced to the left side of the body and a 27 G needle, which had been attached to a tube and a 1 ml syringe was inserted into the bile duct. The injection of the complexes was performed over a period of 4 minutes. Then the needle was retracted from the bile duct and the injection site sealed with a fibrin sealer (Immuno). The abdominal wound was closed with sutures and metal clips. After 30 hours the rat was killed and samples from different lobes of the liver were assayed for luciferase gene expression. The peak activity of luciferase was 19000 light units/mg protein and the calculated overall expression in the total liver was in the range of 2.7.times.10.sup.6 light units.

DEPR:

Human skin biopsies were put into a 6 cm petri dish containing DMEM, 2 mM glutamine, 20% FCS and antibiotics. Then the biopsies were thoroughly minced with a surgical knife and cultured in the presence of 3 ml medium for 5 days. Thereafter the cells were washed with fresh DMEM containing 2 mM glutamine 10% FCS and antibiotics and cultured for further 7 days. After this period of time the cells were trypsinized and subcultured into new petri dishes. When the cells were almost confluent, they were trypsinized again and stored frozen until transfection. For transfection the cells were thawed and seeded into 6 cm petri dishes and cultured in DMEM containing 2 mM glutamine 10% FCS and antibiotics. The transfection conjugates were prepared as follows: 3 .mu.l, 10 .mu.l, 20 .mu.l and 30 .mu.l of biotinylated adenovirus dl312 were incubated with 0.1 .mu.g, 0.3 .mu.g, 0.5 .mu.g and 0.8 .mu.g polylysine-modified streptavidin in 150 .mu.l HBS for 30 minutes at room temperature. Then 6 .mu.g of pCMV-.beta.gal plasmid in 170 .mu.l HBS were added and the mixture was incubated for further 30 minutes. In the final step 7.8 .mu.g TfpL for the conjugates with 3 .mu.l dl312, 7 .mu.g TfpL for 10 .mu.l dl312 and 6 .mu.g TfpL for the conjugates with 20 .mu.l and 30 .mu.l dl312 in 170 .mu.g HBS were added. After an incubation period of 30 minutes the conjugates were applied to the cells in 2 ml DMEM containing 2 mM glutamine, 2% FCS and antibiotics and the cells were incubated for 4 hours at 37.degree. C. Then the medium was removed and culture was continued at 37.degree. C. with DMEM containing 2 mM glutamine, 10% FCS and antibiotics. After 48 hours the expression of .beta.-galactosidase was demonstrated as described in previous Examples.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 26. Document ID: US 6057096 A

L3: Entry 26 of 84

File: USPT

May 2, 2000

DOCUMENT-IDENTIFIER: US 6057096 A

TITLE: Photocleavable agents and conjugates for the detection and isolation of biomolecules

DEPR:

Biotin-streptavidin technology is widely used as the basis for non-radioactive ELISA including diagnostic assays for specific indicators of diseases and disorders such as disease-linked antigens including adenovirus antigen (K. Mortensson-Egnund et al., J. Virol. Methods 14:57, 1986), bovine leukemia virus (E. N. Esteban et al., Cancer Res. 45:3231, 1985), flavivirus (E. A. Gould et al., J. Virol. Methods 11:41, 1985), Hepatitis B surface antigen (C. Kendall et al., J. Immunol. Methods 56:329, 1983), Herpes simplex virus antigen (K. Adler-Strorthz et al., J. Clin. Microbiol. 18:1329, 1983), respiratory syncytial virus (A. Hornsleth et al., J. Med. Virol. 18:113, 1986), bacterial antigens (R. H. Yolken et al., J. Immunol. Methods 56:319, 1983) and melanoma-associated antigens (human) (A. C. Morgan et al., Cancer Res. 43:3155, 1983). The usefulness of these assays can be compromised if endogenous biotin is present in the sample. In this case, a false background will be obtained since the streptavidin-reporter enzyme complex will react both to non-specific biotins and to the biotinylated antibodies directed against the target protein. While several approaches to eliminate background due to non-specific binding of the avidin or streptavidin to non-biotinylated targets including the use of high ionic-strength buffers (C. J. P. Jones et al., Histochem. J. 19:264, 1987), milk proteins (R. C. Duhamel et al., J. Histochem. Cytochem. 33:711, 1985) and lysozyme (E. A. Bayer et al., Anal. Biochem. 163:204, 1987) and altered streptavidins such as Immunopure NeutrAvidin (Pierce Chemical; Rockford, Ill.), none has been very effective in eliminating background due to endogenous biotin.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KMC	Draw Desc	Image
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☐ 27. Document ID: US 6022951 A

L3: Entry 27 of 84

File: USPT

Feb 8, 2000

DOCUMENT-IDENTIFIER: US 6022951 A

TITLE: Streptavidin mutants

DRPR:

FIG. 1 Schematic of expression vector for a streptavidin mutant with a reduced binding affinity.

DRPR:

FIG. 11 Expression of streptavidin mutants Stv-C127, Stv-D127 and Stv-K127.

DEPR:

The mutated streptavidin of Example 1 was used to produce large quantities Phe-120 streptavidin protein. Because the expression of streptavidin in bacteria has a lethal effect to a cell, an inducible system was used. The DNA fragment comprising the sequence encoding the streptavidin mutant was excised from its vector with the restriction endonucleases Nde I and BamH I, and cloned into the same sites in the T7 expression vector pET-3a. Resultant plasmids were transformed in BL21(DE3) (pLysE) bacteria.

DEPR:

Phe-120 streptavidin protein produced by induced E. coli was purified. Cells expressing the mutant streptavidin were harvested by centrifugation at

1600.times.g for 10 minutes. Protein was purified from the insoluble fraction of cell extracts. Briefly, cells were pelleted, washed with an isotonic solution of 100 mM NaCl, 1 mM EDTA and 10 mM Tris, pH 8.0, and resuspended in a detergent solution of 2 mM EDTA, 30 mM Tris-HCl, pH 8.0, 0.1% Triton X-100. Lysis occurred under these conditions because the presence of T7 lysozyme in the cells.

DEPR:

Impurities were removed by dialysis against 6 M guanidine hydrochloride pH 1.5. Mutant streptavidin was renatured slowly by dialysis against 0.2 M ammonium acetate, pH 6. After renaturation, insoluble impurities were removed by centrifugation at 39,000.times.g. Supernatant containing the mutant streptavidin protein was removed and collected. Final purifications were performed by 2-iminobiotin affinity chromatography.

DEPR:

On gel filtration chromatography using a Superdex 75 HR 10/30 column the molecular mass of purified Phe-120 streptavidin was estimated to be 49,000 daltons, consistent with proper tetramer formation. When the biotin binding Phe-120 streptavidin was analyzed, biotin was found at an amount of greater than 0.97 molecules of biotin per streptavidin subunit. This degree of binding is consistent with full biotin-binding ability. These results indicate that Phe-120 streptavidin forms a tetramer as does wild type streptavidin and that the conversion of Trp-120 to Phe-120 has no significant effect on the basic properties of the mutant streptavidin. These data also indicate that the mutation had minimal effects on local environments around the biotin-binding sites and the dimer--dimer interface, thus allowing the correct folding of the molecule.

DEPR:

The biotin-binding affinities of wild type and Phe-120 streptavidin were determined by an equilibrium dialysis method using a micro dialyzer (Hoeffer Scientific). One hundred microliters each of D-[carbonyl-¹⁴C] biotin (2 nM-4 .mu.M; 53 mCi/mmol; Amersham) and 100 .mu.l of streptavidin (5.3 .mu.g/ml, 0.42 .mu.M subunits) were prepared separately in TBS (150 mM NaCl, 20 mM Tris-HCl, pH 7.4, 0.02% NaN.sub.3) solutions. Equilibrium dialysis analysis was begun by the placement of the two solutions into two opposing chambers of a micro dialyzer. Chambers were incubated at 30.degree. C. with rotation for 48 hours and the concentration of labeled biotin in each chamber was measured by scintillation counting. Results were plotted on a Scatchard plot. The apparent biotin-binding affinity of the Phe-120 streptavidin was determined to be from about 1-3.times.10.sup.8 M.sup.-1 demonstrating the reduced substrate affinity of this streptavidin mutant.

DEPR:

Reduced substrate affinity streptavidin was dialyzed extensively against binding buffer (0.5 M sodium phosphate, pH 7.5) to remove inhibitors before attaching to solid supports. Cyanogen bromide activated beads (Pharmacia Biotech; Piscataway, N.J.) were washed with 100 volumes of binding buffer to remove preservatives and used immediately for coupling. Mutant streptavidin was crosslinked to the beads by adding the activated beads to the mutant streptavidin and mixing gently overnight at room temperature. Uncrosslinked mutant streptavidin was removed by washing the beads with binding buffer followed by a solution of 1 M NaCl and 0.05 M sodium phosphate, pH 7.5. Unreacted groups were blocked by incubating the beads in 100 mM ethanolamine, pH 7.5, for 4 hours with gentle mixing. After ethanolamine was removed by washes with phosphate buffered saline (PBS; 0.144 g/L KH.sub.2 PO.sub.4, 9 g/L NaCl, 0.795 g/L Na.sub.2 HPO.sub.4 7H.sub.2 O), the reduced substrate affinity streptavidin coupled beads were ready for use.

DEPR:

An oncogene expression profile of a tumor can be determined by successive probing of a western blot using a variety of antibodies conjugated to .sup.125 I-labeled streptavidin. Briefly, 500 .mu.Ci of .sup.125 I-labeled N-succinimidyl 3-(4-hydroxyphenyl propionate) (ICN Radiochemicals; Irvine,

Calif.) in dimethylformamide is air dried to the bottom of a tube. Radiolabeling is initiated by the addition of 10 .mu.g of reduced substrate affinity streptavidin in 10 .mu.l of 0.1 M sodium borate to the tube. Mutant streptavidin is labeled on ice for 15 minutes and the reaction terminated with 100 .mu.l of 0.5 M ethanolamine, 10% glycerol, 0.1% xylene cyanol and 0.1 M sodium borate, pH 8.5. Labeled streptavidin is separated from the labeling reagent on a gel filtration column.

DEPR:

Expression vectors were constructed by standard methods using pTSA-13 which encodes the minimum sized core streptavidin consisting of amino acid residues 16-133 as a starting material. Oligonucleotide-directed mutagenesis was performed on a bacteriophage M13mpl8 derivative, mpSA-29, carrying the entire coding sequence for the minimum-sized core streptavidin from pTSA-13, to convert the codon for His-127 (CAC) to GAC for Asp using an 18-base oligonucleotide, 5'-d(AGGTG TCGTC GCCGA CCA)-3' (SEQ ID NO 3). A 405-bp Nde I fragment carrying the entire coding sequence was cloned into the Nde I site of the plasmid pET-3a under the control of the psi10 promoter. The resulting expression vector, pTSA-33 (FIG. 6), encodes a core streptavidin mutant, Stv-33 (12.6 kDa per subunit) in which His-127 is replaced with Asp-127.

DEPR:

Two separate polymerase chain reactions (PCR) were performed using the expression vector pTSA-33 as the template to generate two partial DNA fragments of the coding sequence for streptavidin. One PCR amplification used the following set of oligonucleotides as primers: 5'-d(AATAC GACTC ACTAT AG)-3' (T7 promoter primer; SEQ ID NO 4) and 5'-d(GTTG T TCGAA GTCAG CAGCC ACTGG GT)-3' (SEQ ID NO 5). This PCR amplification generated a 380-bp fragment containing a sequence from the translation initiation site to the codon for Ser-112 followed by the recognition sequence for the restriction endonuclease BstB I (underlined). The other PCR amplification used the following set of primers: 5'-d(TTGC T TCGAA GTCCA CGCTG GTCGG C)-3' (SEQ ID NO 6) and 5'-d(CGGGC TTTGT TAGCA GCCGG A)-3' (SEQ ID NO 7). This PCR amplification generated a 150-bp fragment containing a BstB I recognition site (underlined) followed by a sequence from the codon for Lys-121 to a translation termination codon (TAG). The 380-bp PCR fragment was digested with Nde I and BstB I, and the 150-bp PCR fragment was with BamH I and BstB I. The resulting two fragments were ligated via the BstB I termini and the ligated fragment (420-bp) was cloned between the Nde I and BamH I sites of pET-3a under the control of the psi10 promoter. The resulting expression vector, pTSA-43 (FIG. 6), encodes a core streptavidin mutant, Stv-43 (11.8 kDa per subunit) in which a sequence from Gly-113 to Trp-120 has been deleted in addition to the mutation of His-127 to Asp-127. pTSA-43 also carries silent mutations in the codons for Thr-111 (ACC) and Ser-112 (TCC) which have been converted to ACT and TCG, respectively.

DEPR:

Cysteine residues are added to the carboxyl terminus of streptavidin to allow conjugation to other molecules through sulfhydryl reactions. A plasmid DNA, encoding residues 16 to 133 of streptavidin with Lys at position 127 is used as the starting material. Plasmid is digested with EcoR I and BamH I. Two 21 mer oligonucleotides, 5'-d(AAT TGC TGC TGC TGC TGC TAA)-3' (SEQ ID NO 8), 5'-d(GAT CTT AGC AGC AGC AGC AGC)-3' (SEQ ID NO 9) are annealed, and the resulting double-stranded DNA inserted and ligated into the EcoR I and BamH I sites of the predigested plasmid. The sequence of the resulting plasmid is confirmed by DNA sequencing using a dideoxy termination method. This gene is cloned into a bacterial expression vector and the mutated streptavidin expressed and purified. This streptavidin mutant has all the properties of the previous streptavidin mutant. It forms heterotetramers in solution and, with a phenylalanine at position 120, has a reduced biotin-binding affinity of less than about 10.sup.8 M.sup.-1. In addition, this streptavidin mutant may be conjugated to other proteins and macromolecules, and also solid supports through the sulfhydryl group on the cysteines.

DEPR:

Expression vectors were constructed by using standard techniques (J. Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 2nd Edition, 1989). mpSA-29, derived from M13mpl8, which codes for a core streptavidin (E. A. Bayer et al., Biochem. J. 259:369-76, 1989; T. Sano et al., Proc. Natl. Acad. Sci. USA 87:142-46, 1989) containing amino acids 16 to 133 (T. Sano et al., Proc. Natl. Acad. Sci. USA 92:3180-84, 1995) was used as a starting material. Mutations were introduced into the coding sequence of mpSA-29 using an oligonucleotide-directed in vitro mutagenesis system (Amersham) (J. R. Sayers et al., Nucleic Acids Res. 16:791-802, 1988). Three sets of mutations were introduced separately into a codon for His-127 (CAC): CAC.fwdarw.TGC for Cys; CAC.fwdarw.AAA for Lys; and CAC.fwdarw.GAC for Asp. Coding sequences containing the desired mutations were cloned into the Nde I site of pET-3a under the control of psi10 promoter (F. W. Studier et al., Methods Enzymol. 185:60-89, 1990). Resulting expression vectors, pTSA-C127, pTSA-K127 and pTSA-33 encode the streptavidin mutants Stv-C127, Stv-K127 and Stv-D127, in which His-127 is replaced by Cys, Lys or Asp, respectively.

DEPR:

Expression of each streptavidin mutant was carried out as described (T. Sano et al., Proc. Natl. Acad. Sci. USA 87:142-46, 1989) using BL21 (DE3) (pLysE) (F. W. Studier et al., Methods Enzymol. 185:60-89, 1990) carrying an expression vector. Each mutant was purified which included 2-iminobiotin affinity chromatography (K. Hofmann et al., Proc. Natl. Acad. Sci. USA 77:4666-68, 1980). For Stv-C127, 2-mercaptoethanol was included in all solutions to prevent disulfide bond formation during purification (T. Sano et al., Bio/Technology 11:201-6, 1993).

DEPR:

Thermal stability of streptavidin mutants was determined. Each streptavidin construct (approximately 240 pmoles) with or without D-[carbonyl-¹⁴C]biotin (52 mCi/mmol; Amersham) was dissolved in 6.5 μ l of water and heated from 25.degree. C. to 70.degree. C., 80.degree. C., 90.degree. C., and 95.degree. C. at a rate of 2.degree. C./minute. Protein solutions were kept at these temperatures for 10 minutes and cooled to 25.degree. C. at a rate of 2.degree. C./minute. Resulting protein samples were incubated at room temperature for 1 hour in 1.25% SDS, 40 mM Tris-HCl, pH 6.8, and subjected to SDS-PAGE analysis. cl Example 20

DEPR:

Expression vectors were constructed using standard techniques (J. Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989). mpSA-29, derived from M13mpl8, which codes for a core streptavidin (W. A. Hendrickson et al., Proc. Natl. Acad. Sci. USA 86:2190-94, 1989; C. E. Argara na et al., Nucleic Acids Res. 14:1871-82, 1986; Pahler et al., J. Biol. Chem. 262:13933-937, 1987; E. A. Bayer et al., Biochem. J. 259:369-76, 1989; T. Sano et al., Proc. Natl. Acad. Sci. USA 87:142-46, 1989) containing amino acids 16 to 133 as a starting material (T. Sano et al., Proc. Natl. Acad. Sci. USA 92:3180-84, 1995). Mutations were introduced into the coding sequence of mpSA-29 with an oligonucleotide-directed in vitro mutagenesis system (Amersham) (J. R. Sayers et al., Nucleic Acids Res. 16:791-802, 1988). Three sets of mutations were introduced separately into a codon for His-127 (CAC): (i) CAC.fwdarw.TGC for Cys; (ii) CAC.fwdarw.AAA for Lys; and (iii) CAC.fwdarw.GAC for Asp. The coding sequence containing the desired mutations was cloned into the Nde I site of pET-3a under the control of .PHI.10 promoter (F. W. Studier et al., Methods Enzymol. 185:60-89, 1990). The resulting expression vectors, pTSA-C127, pTSA-K127 and pTSA-33 encode the streptavidin mutants Stv-C127, Stv-K127 and Stv-D127, in which His-127 is replaced by Cys, Lys, or Asp, respectively.

DEPR:

Streptavidin mutants were produced in E. coli by using the T7 expression system (F. W. Studier et al., Methods Enzymol. 185:60-89, 1990), as previously described (T. Sano et al., Proc. Natl. Acad. Sci. USA 87:142-46, 1989). The expressed products of BL21(DE3) (pLysE) (pTSA-C127) (FIG. 11 lane C), BL21(DE3) (pLysE) (pTSA-D127) (FIG. 11 lane D), BL21 (DE3) (pLysE) (PTSA-K 1

27) (FIG. 11 lane E), a negative control, BL21 (DE3) (pLysE) (FIG. 11 lane b) and molecular weight markers (FIG. 11 lane A) were analyzed by SDS-PAGE and shown in FIG. 11. Two time points, at zero hours and five hours after induction, were taken for each sample and the arrow indicates the location of expressed streptavidin.

DEPR:

Expression of each streptavidin mutant was carried out as described by Sano and Cantor (Proc. Natl. Acad. Sci. USA 87:142-46, 1989) using BL21 (DE3) (pLysE) carrying an expression vector (F. W. Studier et al., Methods Enzymol. 185:60-89, 1990). Purification of each mutant followed the method of Sano and Cantor (Proc. Natl. Acad. Sci. USA 87:142-46, 1989), which includes 2-iminobiotin affinity chromatography (K. Hofmann et al., Proc. Natl. Acad. Sci. USA 77:4666-68, 1980). For Stv-C127, 2-mercaptoethanol was included in all solutions to prevent disulfide bond formation during purification (T. Sano et al., Bio/Technology 11:201-6, 1993).

DEPL:

Expression and Purification of Streptavidin Mutants.

DEPL:

Stability of Streptavidin Mutants in Guanidine Hydrochloride.

DEPL:

Construction of Streptavidin Mutants.

DEPL:

Expression and Purification of Streptavidin Mutants.

DEPL:

Thermal Stability of Streptavidin Mutants.

DEPL:

Stability of Streptavidin Mutants in Guanidine Hydrochloride.

ORPL:

Sano et al. "A streptavidin mutant containing a cysteine stretch that facilitates production of a variety of specific streptavidin conjugates." Bio/technology 11: 201-206, Feb. 11, 1993.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 28. Document ID: US 6022735 A

L3: Entry 28 of 84

File: USPT

Feb 8, 2000

DOCUMENT-IDENTIFIER: US 6022735 A

TITLE: Composition for introducing nucleic acid complexes into higher eucaryotic cells

BSPR:

The invention also relates to a transfection kit, comprising a carrier means having in close confinement therein one or more container means, wherein a first container means contains a biotin-modified endosomolytic agent and a second container means contains a streptavidin-modified substance having affinity for nucleic acid.

DEPR:

The coupling of streptavidin to polylysine was effected using the method

described by Wagner et al., 1990, and in EP-A1 388 758. 79 nmol (4.7 mg) of streptavidin in 1 ml of 200 mM HEPES pH 7.9 and 300 mM NaCl were treated with a 15 mM ethanolic solution of SPDP (236 nmol). After 1.5 hours at ambient temperature the modified protein was gel filtered over a Sephadex G-25 column, thereby obtaining 75 nmol of streptavidin, modified with 196 nmol of dithiopyridine linker. The modified protein was reacted under an argon atmosphere with 3-mercaptopropionate-modified polylysine (75 nmol, average chain length 290 lysine monomers, modified with 190 nmol mercapto-propionate linker) in 2.6 ml of 100 mM HEPES pH 7.9, 150 mM NaCl. Conjugates were isolated by cation exchange chromatography on a Mono S HR5 column (Pharmacia). (Gradient: 20-100% buffer B. Buffer A: 50 mM HEPES pH 7.9; buffer B: buffer A plus 3 M sodium chloride). The product fraction eluted at a salt concentration of between 1.2 M and 1.7 M. Dialysis against HBS (20 mM HEPES pH 7.3, 150 mM NaCl) resulted in a conjugate consisting of 45 nmol of streptavidin and 53 nmol of polylysine.

DEPR:

For formation of adenovirus-DNA-transferrin complexes 50 .mu.l of biotinylated adenovirus were incubated with 400 ng of streptavidin-modified polylysine in 20 .mu.l HBS for 20 min. Then 20 .mu.l of HBS containing 6 .mu.g pCMVL were added. After an incubation period for 20 min 7 .mu.g of mouse transferrin-polylysine conjugate (mTfpl) in 160 .mu.l HBS were added and the whole mixture was incubated for further 20 min.

DEPR:

The complexes were prepared as follows: 200 .mu.l biotinylated Adenovirus dl312 diluted with 200 .mu.l HBS were incubated with 6.4 .mu.g streptavidin-modified polylysine in 400 .mu.l HBS for 30 minutes at room temperature. Then 48 .mu.g of pCMV-L in 800 .mu.l HBS were added. After 30 minutes of incubation 48 .mu.g of Tfpl in 900 .mu.l HBS were further added. For application of the complexes male Sprague Dawley rats (250 g body weight) were anesthetized with Avertin and the abdomen opened with a median incision. The intestine was displaced to the left side of the body and a 27 G needle, which had been attached to a tube and a 1 ml syringe was inserted into the bile duct. The injection of the complexes was performed over a period of 4 minutes. Then the needle was retracted from the bile duct and the injection site sealed with a fibrin sealer (Immuno). The abdominal wound was closed with sutures and metal clips. After 30 hours the rat was killed and samples from different lobes of the liver were assayed for luciferase gene expression. The peak activity of luciferase was 19000 light units/mg protein and the calculated overall expression in the total liver was in the range of 2.7.times.10.sup.6 light units.

DEPR:

Human skin biopsies were put into a 6 cm petri dish containing DMEM, 2 mM glutamine, 20% FCS and antibiotics. Then the biopsies were thoroughly minced with a surgical knife and cultured in the presence of 3 ml medium for 5 days. Thereafter the cells were washed with fresh DMEM containing 2 mM glutamine 10% FCS and antibiotics and cultured for further 7 days. After this period of time the cells were trypsinized and subcultured into new petri dishes. When the cells were almost confluent, they were trypsinized again and stored frozen until transfection. For transfection the cells were thawed and seeded into 6 cm petri dishes and cultured in DMEM containing 2 mM glutamine 10% FCS and antibiotics. The transfection conjugates were prepared as follows: 3 .mu.l, 10 .mu.l, 20 .mu.l and 30 .mu.l of biotinylated adenovirus dl312 were incubated with 0.1 .mu.g, 0.3 .mu.g, 0.5 .mu.g and 0.8 .mu.g polylysine-modified streptavidin in 150 .mu.l HBS for 30 minutes at room temperature. Then 6 .mu.g of pCMV-.beta.gal plasmid in 170 .mu.l HBS were added and the mixture was incubated for further 30 minutes. In the final step 7.8 .mu.g Tfpl for the conjugates with 3 .mu.l dl312, 7 .mu.g Tfpl for 10 .mu.l dl312 and 6 .mu.g Tfpl for the conjugates with 20 .mu.l and 30 .mu.l dl312 in 170 .mu.g HBS were added. After an incubation period of 30 minutes the conjugates were applied to the cells in 2 ml DMEM containing 2 mM glutamine, 2% FCS and antibiotics and the cells were incubated for 4 hours at 37.degree. C. Then the medium was removed and culture was continued at 37.degree. C. with DMEM containing 2 mM

glutamine, 10% FCS and antibiotics. After 48 hours the expression of .beta.-galactosidase was demonstrated as described in previous Examples.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 29. Document ID: US 6015897 A

L3: Entry 29 of 84

File: USPT

Jan 18, 2000

DOCUMENT-IDENTIFIER: US 6015897 A

TITLE: Biotinamido-n-methylglycyl-seryl-o-succinamido-benzyl dota

DEPR:

Biotin binding capacity of the PEG derivatized streptavidin molecule was then determined using the displacement of 2-(4'-hydroxy-azobenzene)benzoic acid (HABA) from streptavidin by biotin. When HABA is bound to streptavidin there is a spectral shift at 500 nm which is proportional to the amount of bound HABA. HABA is quantitatively displaced from streptavidin by biotin. This enables the titration of the HABA-SA complex with biotin to be monitored directly by 500 nm absorbance, thereby providing a molar concentration of biotin binding sites. The ratio of binding site concentration to modified streptavidin concentration provides an indication of how the modification has disturbed biotin binding ability. (Unmodified streptavidin binds four biotin molecules.) The experimental protocol is as follows:

DEPR:

Subsequent experiments validated the utility of this method and the inventors' initial hypotheses. In vivo and in vitro experiments showed that streptavidin modified with PEG in the manner described herein reduced its immunogenicity to background levels. Also, the streptavidin derivatized with PEG in this manner was readily conjugated to antibody for antigen mediated targeting. Thus, these results demonstrate that polyethylene glycol derivatized ligands and anti-ligands, e.g., streptavidin and avidin, are useful in the preparation of conjugates for use in pretargeting methods. Such PEG derivatized ligands and anti-ligands are especially suitable for therapeutic pretargeting methods where immunogenicity may be a potential concern.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 30. Document ID: US 6007987 A

L3: Entry 30 of 84

File: USPT

Dec 28, 1999

DOCUMENT-IDENTIFIER: US 6007987 A
TITLE: Positional sequencing by hybridization

DEPR:

Manipulation of DNA in the solid state. Complexes between streptavidin (or avidin) and biotin represent the standard way in which much solid state DNA sequencing or other DNA manipulation is done, and one of the standard ways in which non-radioactive detection of DNA is carried out. Over the past few years streptavidin-biotin technology has expanded in several ways. Several years ago, the gene for streptavidin was cloned and sequenced (C. E. Argarana et al., Nuc. Acids Res. 14:1871, 1986). More recently, using the Studier T7 system, over-expression of the Protein in E. coli was achieved (T. Sano and C. R. Cantor, Proc. Natl. Acad. Sci. USA 87:142, 1990). In the last year, mutant streptavidins modified for improved solubility properties and firmer attachment to solid supports was also expressed (T. Sano and C. R. Cantor, Bio/Technology 9:1378-81, 1993). The most relevant of these is core streptavidin, (fully active protein with extraneous N- and C-terminal peptides removed) with 5 cysteine residues attached to the C-terminus. An active protein fusion of streptavidin to two IgG binding domains of staphylococcal A protein was also produced (T. Sano and C. R. Cantor, Bio/Technology 9:1378-81, 1991). This allowed biotinylated DNAs to be attached to specific Immunoglobulin G molecules without the need for any covalent chemistry, and it has led to the development of immuno-PCR, an exceedingly sensitive method for detecting antigens (T. Sano et al., Sci. 258:120-29, 1992).

Full	Title	Citation	Front	Review	Classification	Date	Reference
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RWMC	Draw Desc	Image
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☐ 31. Document ID: US 5998588 A

L3: Entry 31 of 84

File: USPT

Dec 7, 1999

DOCUMENT-IDENTIFIER: US 5998588 A
TITLE: Interactive molecular conjugates

DEPR:

To facilitate placement of a stimulus-responsive component attachment site in or near the binding pocket or other desired site, site-directed binding pocket mutants can be produced to optimize the thermodynamic and kinetic properties specifically for use with stimulus-responsive components. Such mutants display altered ligand affinities and thus provide additional control over protein function for optimizing stimulus-responsive component-molecular conjugates. Libraries of site-directed affinity mutants can be used. For example, several ligand-binding site mutations have been constructed in streptavidin which alter important biotin-binding interactions, as described in copending U.S. application Ser. No. 08/387,055, incorporated herein by reference. This provides a choice for the initial ligand-binding thermodynamic properties, so that changes in affinity triggered by the stimulus-responsive component can be engineered to fall in a useful and desired range.

DEPR:

A unique cysteine thiol group was introduced near the binding pocket of streptavidin, which has no native cysteine residues, by substituting Cys for Asn at position 49, which is hydrogen-bonded to the terminal carboxylate of the valeric acid hydrocarbon side-chain of biotin. Cassette site-directed mutagenesis methods were used to create the N49C mutant streptavidin, which places the engineered polymer conjugation site near the outer edge of the biotin-binding pocket.

DEPR:

The N49C site-directed streptavidin mutant was constructed by cassette mutagenesis techniques, using a synthetic "core" streptavidin gene designed for bacterial expression. The streptavidin gene was cut between the Kpn I and Xba I sites, and a synthetic cassette introducing the desired Asn to Cys codon change was subsequently ligated between the sites. The N49C streptavidin mutant sequence was confirmed by automated dideoxy sequencing and subclones into pET 11 (Novagen, Madison, Wis.) for expression in E. coli strain BL21(DE3). The over-expressed protein was refolded using protocols of Stayton, supra, with the addition of 0.5 mM DTT to maintain the thiol oxidation state.

DEPR:

The poly(NIPAAm)-streptavidin conjugate was next immobilized on a biotinylated microporous membrane. Hydrophilic poly(vinylidene difluoride) (PVDF) membranes (Millipore, Bedford, Mass.) were first coated with polyethyleneimine (PEI), a primary amine-containing polymer, by physical adsorption of 50 kD PEI. A biotinylated surface was then prepared by conjugating the N-hydroxy succinimidyl ester of biotin to the PEI primary amine groups. The N49C streptavidin-poly(NIPAAm) conjugate was adsorbed by affinity binding of the streptavidin to the biotinylated membrane in 100 mM phosphate buffer (pH 7.0). As controls, wild-type streptavidin and the N49C streptavidin mutant were also adsorbed at comparable loading densities (as quantitated by 35 S biotin binding to the adsorbed streptavidin surfaces) to the conjugate-coated biotinylated membranes. The 35 S biotin-binding capacity of the membranes were then measured in triplicates at 4.degree. C. and 37.degree. C. At 4.degree. C. the poly (NIPAAm) is below its 32.degree. C. phase transition temperature, and the polymer is a hydrated coil. At this temperature, the biotin-binding capacity of the N49C streptavidin conjugate on the membrane was essentially identical to the wild-type or N49C mutant streptavidin controls. However, a sharp decrease in the biotin-binding capacity of the conjugate on the membrane was observed when the temperature was raised to 37.degree. C., where the polymer is a collapsed globule. The wild-type and N49C streptavidin controls did not show any significant temperature-dependent biotin binding capacity. An 84% drop in biotin association for the conjugate was observed at 37.degree. C. relative to 4.degree. C., while the wild-type control exhibited only a 3% drop in biotin-binding capacity at 37.degree. C. vs. 4.degree. C.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KOMC	Draw Desc	Image
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☐ 32. Document ID: US 5986076 A

L3: Entry 32 of 84

File: USPT

Nov 16, 1999

DOCUMENT-IDENTIFIER: US 5986076 A

TITLE: Photocleavable agents and conjugates for the detection and isolation of biomolecules

DEPR:

Biotin-streptavidin technology is widely used as the basis for nonradioactive EUSA including diagnostic assays for specific indicators of diseases and disorders such as disease-linked antigens including adenovirus antigen (K Mortensson-Egnund et al., J. Virol. Methods 14:57, 1986), bovine leukemia virus (E. N. Esteban et al., Cancer Res. 45:3231, 1985), flavivirus (E. A. Gould et al., J. Virol. Methods 11:41, 1985), Hepatitis B surface antigen (C. Kendall et al., J. Immunol. Methods 56:329, 1983), Herpes simplex virus antigen (K. Adler-Strorthz et al., J. Clin. Microbiol. 18:1329, 1983), respiratory syncytial virus (A. Hornsleth et al., J. Med. Virol. 18:113, 1986), bacterial antigens (R. H. Yolken et al., J. Immunol. Methods 56:319, 1983) and melanoma-associated antigens (human) (A. C. Morgan et al., Cancer Res. 43:3155, 1983). The usefulness of these assays can be compromised if endogenous biotin is present in the sample. In this case, a false background will be obtained since the streptavidin-reporter enzyme complex will react both to non-specific biotins and to the biotinylated antibodies directed against the target protein. While several approaches to eliminate background due to non-specific binding of the avidin or streptavidin to non-biotinylated targets including the use of high ionic-strength buffers (C. J. P. Jones et al., Histochem. J. 19:264, 1987), milk proteins (R. C. Duhamel et al., J. Histochem. Cytochem. 33:711, 1985) and lysozyme (E. A. Bayer et al., Anal. Biochem. 163:204, 1987) and altered streptavidins such as ImmunoPure NeutrAvidin (Pierce Chemical; Rockford, Ill.), none has been very effective in eliminating background due to endogenous biotin.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KMOC	Draw Desc	Image
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☐ 33. Document ID: US 5986061 A

L3: Entry 33 of 84

File: USPT

Nov 16, 1999

DOCUMENT-IDENTIFIER: US 5986061 A
TITLE: Phosphorylated fusion proteins

BSPR:

The invention also provides particularly interesting labellable and labelled proteins like phosphorylated antibodies (especially monoclonal antibodies, hybrid antibodies, chimeric antibodies or modified antibodies), hormones, and "modified" streptavidin. The modified streptavidin can be bound to individual biotinylated antibodies, each streptavidin being modified by single or multiple phosphorylated groups, which product has greatly enhanced radiation and therefore diagnostic and therapeutic potential.

DEPR:

A particularly noteworthy and interesting application made possible by the invention is what has been called here in the vernacular, a therapeutic or more specifically an antitumor "therapeutic radiation bomb". Such a biologically-active composition uses biotin coupled to a tumor-specific monoclonal antibody (Mab) (or to Fab or Fab' fragments if more appropriate), and a multiple "modified" streptavidin bound to each Mab-bound biotin, each streptavidin being modified in that it has multiple phosphorylated groups. Since streptavidin is itself a tetramer, multiple radioactive groups are thus provided. These multiple radioactive groups expose the tumor with radiation which is greatly amplified and hence more readily detectable and would produce greater tumor destruction. In the case where it is highly phosphorylatable it is much more easily detectable. Thus, each one of the biotins which is bound to each tumor-specific Mab binds tightly to the multiple streptavidin molecules which in turn contain multiple labelled phosphorus atoms, or their equivalent isotopes.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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RIMC	Draw Desc	Image
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☐ 34. Document ID: US 5981273 A

L3: Entry 34 of 84

File: USPT

Nov 9, 1999

DOCUMENT-IDENTIFIER: US 5981273 A
TITLE: Composition comprising an endosomolytic agent for introducing nucleic acid complexes into higher eucaryotic cells

BSPR:

The invention also relates to a transfection kit, comprising a carrier means having in close confinement therein one or more container means, wherein a first container means contains a biotin-modified endosomolytic agent and a second container means contains a streptavidin-modified substance having affinity for nucleic acid.

DEPR:

The coupling of streptavidin to polylysine was effected using the method described by Wagner et al., 1990, and in EP-A1 388 758. 79 nmol (4.7 mg) of streptavidin in 1 ml of 200 mM HEPES pH 7.9 and 300 mM NaCl were treated with a 15 mM ethanolic solution of SPDP (236 nmol). After 1.5 hours at ambient temperature the modified protein was gel filtered over a Sephadex G-25 column, thereby obtaining 75 nmol of streptavidin, modified with 196 nmol of dithiopyridine linker. The modified protein was reacted under an argon atmosphere with 3-mercaptopropionate-modified polylysine (75 nmol, average chain length 290 lysine monomers, modified with 190 nmol mercapto-propionate linker) in 2.6 ml of 100 mM HEPES pH 7.9, 150 mM NaCl. Conjugates were

isolated by cation exchange chromatography on a Mono S HR5 column (Pharmacia). (Gradient: 20-100% buffer B. Buffer A: 50 mM HEPES pH 7.9; buffer B: buffer A plus 3 M sodium chloride). The product fraction eluted at a salt concentration of between 1.2 M and 1.7 M. Dialysis against HBS (20 mM HEPES pH 7.3, 150 mM NaCl) resulted in a conjugate consisting of 45 nmol of streptavidin and 53 nmol of polylysine.

DEPR:

For formation of adenovirus-DNA-transferrin complexes 50 .mu.l of biotinylated adenovirus were incubated with 400 ng of streptavidin-modified polylysine in 20 .mu.l HBS for 20 min. Then 20 .mu.l of HBS containing 6 .mu.g pCMVL were added. After an incubation period for 20 min 7 .mu.g of mouse transferrin-polylysine conjugate (mTfpL) in 160 .mu.l HBS were added and the whole mixture was incubated for further 20 min.

DEPR:

The complexes were prepared as follows: 200 .mu.l biotinylated Adenovirus dl312 diluted with 200 .mu.l HBS were incubated with 6.4 .mu.g streptavidin-modified polylysine in 400 .mu.l HBS for 30 minutes at room temperature. Then 48 .mu.g of pCMV-L in 800 .mu.l HBS were added. After 30 minutes of incubation 48 .mu.g of TfpL in 900 .mu.l HBS were further added. For application of the complexes male Sprague Dawley rats (250 g body weight) were anesthetized with Avertin and the abdomen opened with a median incision. The intestine was displaced to the left side of the body and a 27 G needle, which had been attached to a tube and a 1 ml syringe was inserted into the bile duct. The injection of the complexes was performed over a period of 4 minutes. Then the needle was retracted from the bile duct and the injection site sealed with a fibrin sealer (Immuno). The abdominal wound was closed with sutures and metal clips. After 30 hours the rat was killed and samples from different lobes of the liver were assayed for luciferase gene expression. The peak activity of luciferase was 19000 light units/mg protein and the calculated overall expression in the total liver was in the range of 2.7.times.10.sup.6 light units.

DEPR:

Human skin biopsies were put into a 6 cm petri dish containing DMEM, 2 mM glutamine, 20% FCS and antibiotics. Then the biopsies were thoroughly minced with a surgical knife and cultured in the presence of 3 ml medium for 5 days. Thereafter the cells were washed with fresh DMEM containing 2 mM glutamine 10% FCS and antibiotics and cultured for further 7 days. After this period of time the cells were trypsinized and subcultured into new petri dishes. When the cells were almost confluent, they were trypsinized again and stored frozen until transfection. For transfection the cells were thawed and seeded into 6 cm petri dishes and cultured in DMEM containing 2 mM glutamine 10% FCS and antibiotics. The transfection conjugates were prepared as follows: 3 .mu.l, 10 .mu.l, 20 .mu.l and 30 .mu.l of biotinylated adenovirus dl312 were incubated with 0.1 .mu.g, 0.3 .mu.g, 0.5 .mu.g and 0.8 .mu.g polylysine-modified streptavidin in 150 .mu.l HBS for 30 minutes at room temperature. Then 6 .mu.g of pCMV-.beta.gal plasmid in 170 .mu.l HBS were added and the mixture was incubated for further 30 minutes. In the final step 7.8 82 g TfpL for the conjugates with 3 .mu.l dl312, 7 .mu.g TfpL for 10 .mu.l dl312 and 6 .mu.g TfpL for the conjugates with 20 .mu.l and 30 .mu.l dl312 in 170 .mu.g HBS were added. After an incubation period of 30 minutes the conjugates were applied to the cells in 2 ml DMEM containing 2 mM glutamine, 2% FCS and antibiotics and the cells were incubated for 4 hours at 37.degree. C. Then the medium was removed and culture was continued at 37.degree. C. with DMEM containing 2 mM glutamine, 10% FCS and antibiotics. After 48 hours the expression of .beta.-galactosidase was demonstrated as described in previous Examples.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KMCC	Draw Desc	Image
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☐ 35. Document ID: US 5973124 A

L3: Entry 35 of 84

File: USPT

Oct 26, 1999

DOCUMENT-IDENTIFIER: US 5973124 A

TITLE: Modified avidin and streptavidin molecules and use thereof

DEPR:

The term "avidin-type molecule" as used herein refers to the native egg-white glycoprotein avidin, to deglycosylated forms of avidin, to bacterial streptavidins produced by selected strains of Streptomyces, e.g., Streptomyces avidinii, to truncated streptavidins, and to recombinant avidin and streptavidin as well as to derivatives of native, deglycosylated and recombinant avidin and of native, recombinant and truncated streptavidin, which are modified at sites other than the essential tyrosine, for example, N-acyl avidins, e.g., N-acetyl, N-phthalyl and N-succinyl avidin, and the commercial products ExtrAvidin.TM. and Neutralite Avidin.TM..

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 36. Document ID: US 5968745 A

L3: Entry 36 of 84

File: USPT

Oct 19, 1999

DOCUMENT-IDENTIFIER: US 5968745 A

TITLE: Polymer-electrodes for detecting nucleic acid hybridization and method of use thereof

DEPR:

Streptavidin is modified with multiple 3'-aminohexylpolyguanine oligonucleotides using glutaraldehyde-mediated covalent coupling. A synthetic biotin derivative bearing a pendant amino group is conjugated to the oxidized carboxylated CYCLOPORE.TM. (PET) membrane via a carbodiimide reaction, and the polymer membrane is affixed to the ITO conductive surface. There is little catalytic current for the biotinylated polymer-electrode. The biotinylated polymer membrane is exposed to poly[dG]-labeled streptavidin. The polymer membrane is washed, contacted to the ITO conductive surface and equilibrated with Ru(bpy).sub.3.sup.2+. Significant catalytic current is measured in response to the amount of labeled streptavidin bound to the polymer membrane.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 37. Document ID: US 5965404 A

L3: Entry 37 of 84

File: USPT

Oct 12, 1999

DOCUMENT-IDENTIFIER: US 5965404 A

TITLE: Method for introducing nucleic acids into higher eukaryotic cells

DEPV:

a) 0.4 .mu.g of streptavidin-modified polylysine was diluted in 75 .mu.l of HBS and incubated for 30 minutes after the addition of 3 .mu.g of pCMVL in 75 .mu.l of HBS. Then 3 .mu.g of transferrin-polylysine (TfpL290) in 75 .mu.l HBS was added and incubation was continued for a further 30 minutes. After the addition of the amount of glycerol as specified in FIG. 1., 700 .mu.l of medium (RPMI 1640 plus FCS, 2mM L-Glu, 1 mM sodium pyruvate, antibiotics) was added and the mixture was pipetted onto the cells. The transfection medium was suction filtered after 4 hours and the cells were mixed with fresh medium (without transfection components). The harvesting of the cells and measurement of the luciferase activity were carried out after 24 hours using standard procedures. The results of the transfections are given in FIG. 1 (the protein quantity of the cell lysate determined according to Bradford is given). Increasing quantities of glycerol in the medium caused an increase in the luciferase activity up to the concentration of 10% (1.15M). At a glycerol content of 15% a drop in expression was observed.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 38. Document ID: US 5958701 A

L3: Entry 38 of 84

File: USPT

Sep 28, 1999

DOCUMENT-IDENTIFIER: US 5958701 A

TITLE: Method for measuring intramolecular forces by atomic force

DEPR:

A 511-bp PCR fragment was amplified from human genomic DNA using a 5'-biotinylated "proximal" primer and 5'-amino-modified "distal" primer. The fragment was purified by double ethanol precipitation and then the distal amine was converted to a thiol using 2-iminothiolane (Traut's reagent, Pierce). This reaction was performed for 30 minutes in a 50 mM triethanolamine buffer at pH 8.0 with 65 mM Traut's reagent at room temperature. The DNA concentration in the reaction mixture was 0.1 to 1 .mu.M. The reaction was quenched by addition of EDTA to 2.0 mM final concentration. The excess of Traut's reagents and free thiols was eliminated by ultrafiltration with Microcon 100 microconcentrators (Millipore) or by multiple ethyl acetate extraction. By a similar procedure, streptavidin was modified to add thiol groups. The streptavidin concentration in the reaction mixture was 6 .mu.M.

CLPR:

12. The method of claim 11 including step (h) of controlling the relative position and orientation of the cantilever and the sample support member to apply sufficient force to the molecule of the modified streptavidin compound and the molecule of the sample compound to rupture the biotin/streptavidin complex and wherein steps (f), (g), and (h) are repeated so that the biotin moiety on a different molecule of the sample compound on the sample support member binds with a different molecule of the modified streptavidin compound on the cantilever.

CLPV:

(c) providing a modified streptavidin compound having at least one thiol functional group attached thereto,

CLPV.

(e) incubating the modified streptavidin compound with the cantilever so that the thiol functional group of the modified streptavidin compound adheres to the gold-plated surface region of the cantilever, thereby immobilizing molecules of the modified streptavidin compound on the surface region of the free end of the cantilever,

CLPV:

(f) controlling the relative position and orientation of the cantilever and the sample support member to select a particular protrusion and to allow a molecule of the modified streptavidin compound and a biotin moiety of a molecule of the sample compound to bind to form a biotin/streptavidin complex, and

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KMOC	Draw Desc	Image
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☐ 39. Document ID: US 5948624 A

L3: Entry 39 of 84

File: USPT

Sep 7, 1999

DOCUMENT-IDENTIFIER: US 5948624 A

TITLE: Methods for the detection and isolation of biomolecules

DEPR:

Biotin-streptavidin technology is widely used as the basis for non-radioactive ELISA including diagnostic assays for specific indicators of diseases and disorders such as disease-linked antigens including adenovirus antigen (K. Mortensson-Egnund et al., J. Virol. Methods 14:57, 1986), bovine leukemia virus (E. N. Esteban et al., Cancer Res. 45:3231, 1985), flavivirus (E. A. Gould et al., J. Virol. Methods 11:41, 1985), Hepatitis B surface antigen (C. Kendall et al., J. Immunol. Methods 56:329, 1983), Herpes simplex virus antigen (K. Adler-Strorthz et al., J. Clin. Microbiol. 18:1329, 1983), respiratory syncytial virus (A. Hornsleth et al., J. Med. Virol. 18:113, 1986), bacterial antigens (R. H. Yolken et al., J. Immunol. Methods 56:319, 1983) and melanoma-associated antigens (human) (A. C. Morgan et al., Cancer Res. 43:3155, 1983). The usefulness of these assays can be compromised if endogenous biotin is present in the sample. In this case, a false background will be obtained since the streptavidin-reporter enzyme complex will react both to non-specific biotins and to the biotinylated antibodies directed against the target protein. While several approaches to eliminate background due to non-specific binding of the avidin or streptavidin to non-biotinylated targets including the use of high ionic-strength buffers (C. J. P. Jones et al., Histochem. J. 19:264, 1987), milk proteins (R. C. Duhamel et al., J. Histochem. Cytochem. 33:711, 1985) and lysozyme (E. A. Bayer et al., Anal. Biochem. 163:204, 1987) and altered streptavidins such as ImmunoPure NeutrAvidin (Pierce Chemical; Rockford, Ill.), none has been very effective in eliminating background due to endogenous biotin.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KMOC	Draw Desc	Image
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☐ 40. Document ID: US 5869348 A

L3: Entry 40 of 84

File: USPT

Feb 9, 1999

DOCUMENT-IDENTIFIER: US 5869348 A

TITLE: Methods for the preparation of antibodies directed against dithiocarbamates and the use thereof for detection of nitric oxide in body fluids

BSPR:

Biotin can also be employed as a principal labeling agent. Several molecules of biotin can be chemically linked to one antibody molecule (Gretch et al., Anal. Biochem., 163:270-277 (1987); Warnke et al., J. Histochem. and Cytochem., 28:771-776 (1980); O'Shannessy et al., Immunol. Lett., 8:273-277 (1984)). When biotin is the principal label, additional reagents such as avidin, streptavidin, or modified avidin are required for production of a signal. Avidin and streptavidin each have 4 subunits and each subunit can bind one molecule of biotin. (Biotin binds with high affinity to avidin and related proteins.) The additional reagent is itself covalently linked to another label such as an enzyme or fluorochrome.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 41. Document ID: US 5863740 A

L3: Entry 41 of 84

File: USPT

Jan 26, 1999

DOCUMENT-IDENTIFIER: US 5863740 A

TITLE: Interference eliminating agent for application in immunoassays

DEPC:

Production of covalently-modified streptavidin

CLPR:

23. A process for the production of an interference eliminating agent, comprising modifying avidin or streptavidin such that said avidin or streptavidin cannot be incorporated into a complex formed between an analyte and a specific binding partner for said analyte, wherein a photoactivatable biotin derivative is bound to the active center and is additionally bound outside the active center via a covalent bond, wherein said avidin or streptavidin is modified by saturating the active center with a photoactivatable biotin derivative and photoactivatable biotin is additionally bound outside the active center of said avidin or streptavidin via a covalent bond and thereafter covalently coupling the biotin derivative by initiating the photoreaction.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 42. Document ID: US 5861254 A

L3: Entry 42 of 84

File: USPT

Jan 19, 1999

DOCUMENT-IDENTIFIER: US 5861254 A
TITLE: Flow cell SELEX

DEPR:

The method of the present invention offers an advantage over soluble target SELEX in that the solubility properties of the target are not an issue in determining whether the SELEX method can be used to find a nucleic acid ligand to a target. This is because the target is immobilized on the sensor chip rather than solubilized in the SELEX buffer solution. One type of sensor chip available comprises a carboxymethylated dextran matrix. A wide range of chemical techniques known in the art can be used to immobilize the target to the dextran matrix, depending on the chemical nature of the target. For example, the target can be directly coupled the matrix through methods such as amine coupling, thiol coupling, or aldehyde coupling. Another type of sensor chip available is one where the matrix has been modified with streptavidin for direct immobilization of biotinylated targets. Furthermore, sensor chips are available which have a hydrophobic surface, and these can be used to immobilize lipophilic substances, such as liposomes, which include a ligand contained in the lipid bilayer of the liposome. Thus, the method of the present invention allows for the identification of nucleic acid ligands to targets which were previously very difficult to use under conditions used in the conventional SELEX method due to difficulties in solubilizing the target in the SELEX solutions.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 43. Document ID: US 5849878 A

L3: Entry 43 of 84

File: USPT

Dec 15, 1998

DOCUMENT-IDENTIFIER: US 5849878 A
TITLE: Design and synthesis of bispecific reagents: use of double stranded DNAs as chemically and spatially defined cross-linkers

ORPL:

Sano, T., et al., OA Streptavidin Mutant Containing a Cysteine Stretch That Facilitates Production of a Variety of Specific Streptavidin Conjugates, 1 Bio/Technology, 11:201-206 (1993).

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 44. Document ID: US 5846537 A

L3: Entry 44 of 84

File: USPT

Dec 8, 1998

DOCUMENT-IDENTIFIER: US 5846537 A

TITLE: Modified avidin and streptavidin and methods of use thereof

ABPL:

The present invention provides modified avidin and streptavidin compounds that have suitable blood clearance kinetics for use in a two-step approach to deliver a molecule to a target site. In particular, to hasten SA's blood clearance carbohydrate moieties are covalently bonded to SA. To prolong Avid's blood clearance, Avid is deglycosylated and/or neutralized by alkylation of its lysine amino acids. In a two-step approach, biotinylated compounds are used to deliver radionuclides, cytotoxic drugs, MRI agents, fluorochromes and other agents suitable for imaging and therapy to target-bound modified streptavidin or avidin conjugated antibodies or other targeting agents.

BSPR:

The present invention provides modified avidin and streptavidin compounds that have optimal blood clearance kinetics for use in a two-step approach to deliver a molecule to a target site. In particular, to hasten streptavidin's blood clearance, carbohydrate moieties are covalently bonded to streptavidin. To prolong avidin's blood clearance, avidin is deglycosylated and/or neutralized by alkylation of its lysine amino acids. The present invention further provides targeting agents, such as monoclonal antibodies, conjugated to modified streptavidin and avidin. In a two-step method of imaging or therapy, biotin conjugates are used to deliver radionuclides, cytotoxic drugs, magnetic resonance imaging agents, fluorochromes and other agents suitable for imaging and therapy to target-bound conjugates of modified streptavidin or avidin and antibodies or other targeting agents.

DEPR:

This example provides the synthesis and characterization of modified streptavidin containing covalently bonded galactose moieties. Galactose was covalently bonded to SA via a nucleophilic reaction of the amino groups of SA with .alpha.-D-galactopyranosyl-phenylisothiocyanate.

CLPR:

1. A conjugate of a targeting agent and a covalently modified streptavidin, wherein said covalently modified streptavidin contains at least one carbohydrate moiety, said carbohydrate moiety being galactose, mannose, fructose or lactose which is covalently attached to the streptavidin.

CLPR:

4. A conjugate of a trigalactose modified streptavidin and a monoclonal antibody, said trigalactose-modified streptavidin (SA) having the formula ##STR5## wherein n is 1 to 20 and gal is galactosyl.

CLPR:

15. The conjugate of claim 1 wherein the covalently modified streptavidin is trigalactose modified streptavidin of the formula ##STR6## wherein n is 1 to 20 and gal is ##STR7##

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 45. Document ID: US 5837533 A

L3: Entry 45 of 84

File: USPT

Nov 17, 1998

DOCUMENT-IDENTIFIER: US 5837533 A

TITLE: Complexes comprising a nucleic acid bound to a cationic polyamine having an endosome disruption agent

BSPR:

Macrophages have receptors for both mannose and mannose-6-phosphate which can bind to and internalize molecules displaying these sugars. The molecules are internalized by endocytosis into a pre-lysosomal endosome. This internalization has been used to enhance entry of oligonucleotides into macrophages using bovine serum albumin modified with mannose-6-phosphate and linked to an oligodeoxynucleotide by a disulfide bridge to a modified 3' end; see E. Bonfils, C. Depierreux, P. Midoux, N. T. Thuong, M. Monsigny and A. C. Roche, Nucl. Acids Res. 20, 4621-4629 (1992). Similarly, oligodeoxynucleotides modified at the 3' end with biotin were combined with mannose-modified streptavidin, and were also found to have facilitated entry into macrophages; see E. Bonfils, C. Mendes, A. C. Roche, M. Monsigny and P. Midoux, Bioconj. Chem., 3, 277-284 (1992).

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 46. Document ID: US 5795714 A

L3: Entry 46 of 84

File: USPT

Aug 18, 1998

DOCUMENT-IDENTIFIER: US 5795714 A

TITLE: Method for replicating an array of nucleic acid probes

DEPR:

Manipulation of DNA in the solid state. Complexes between streptavidin (or avidin) and biotin represent the standard way in which much solid state DNA sequencing or other DNA manipulation is done, and one of the standard ways in which non-radioactive detection of DNA is carried out. Over the past few years streptavidin-biotin technology has expanded in several ways. Several years ago, the gene for streptavidin was cloned and sequenced (C. E. Argarana et al., Nuc. Acids Res. 14:1871, 1986). More recently, using the Studier T7 system, over-expression of the Protein in E. coli was achieved (T. Sano and C. R. Cantor, Proc. Natl. Acad. Sci. USA 87:142, 1990). In the last year, mutant streptavidins modified for improved solubility properties and firmer attachment to solid supports was also expressed (T. Sano and C. R. Cantor, Bio/Technology 9:1378-81, 1993). The most relevant of these is core streptavidin, (fully active protein with extraneous N- and C-terminal peptides removed) with 5 cysteine residues attached to the C-terminus. An active protein fusion of streptavidin to two IgG binding domains of staphylococcal A protein was also produced (T. Sano and C. R. Cantor, Bio/Technology 9:1378-81, 1991). This allowed biotinylated DNAs to be attached to specific Immunoglobulin G molecules without the need for any covalent chemistry, and it has led to the development of immuno-PCR, an exceedingly sensitive method for detecting antigens (T. Sano et al., Sci. 258:120-29, 1992).

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 47. Document ID: US 5747352 A

L3: Entry 47 of 84

File: USPT

May 5, 1998

DOCUMENT-IDENTIFIER: US 5747352 A

TITLE: Reagents and methods for the rapid and quantitative assay of pharmacological agents

DEPR:

The above-described digoxigenin-biotin bidentate was used to define a homogeneous immunoassay for digoxin. The immunoassay is based on the immunoprecipitin reaction and can be monitored by nephelometry or turbidimetry. The assay employs avidin, streptavidin, modified avidin or avidin preferably attached to carrier material (i.e., particles, macromolecules, colloidal metals, colloidal metal oxides). The details of the synthesis of the bidentate molecule and the immunoassay are described below.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 48. Document ID: US 5726012 A

L3: Entry 48 of 84

File: USPT

Mar 10, 1998

DOCUMENT-IDENTIFIER: US 5726012 A

TITLE: Rapid, high capacity nucleic acid based assay

BSPR:

A further variation has been described in German Preliminary Published Application 3,546,312 A1. This method, like that described by Ranki et al., employs a capture probe and a reporter probe which hybridize to distinct portions of the target nucleic acid. The target nucleic acid is contacted in solution by the two probes. The first or capture probe contains a binding component, such as biotin, that is capable of binding with a receptor component, such as streptavidin, which has been affixed to a solid support. After formation of the capture probe--target nucleic acid-reporter probe complex, a streptavidin-modified solid support is added. Any unhybridized reporter probe is washed away followed by the detection of the label incorporated into the complex bound to the solid support.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 49. Document ID: US 5720928 A

L3: Entry 49 of 84

File: USPT

Feb 24, 1998

DOCUMENT-IDENTIFIER: US 5720928 A

TITLE: Image processing and analysis of individual nucleic acid molecules

DEPR:

In another embodiment, detection is based on fluorescent beads and on chemiluminescent tagging using alkaline phosphatase. Single fluorescent beads are easily imaged with fluorescence microscopy, including the smallest ones with a diameter of just 0.01 microns. (Although exceeding the Rayleigh limit, this bead appears as a bright spot.) Fluorescent beads provide a good way to label single DNA molecules for image processing purposes because individual beads are intensely fluorescent, morphologically distinctive, available in wide range of fluorochromes of differing spectral qualities, and are easily attached to oligonucleotides. For example, Molecular Probes, Inc., sells latex beads with coatings of carboxylate, avidin or streptavidin in 6 spectral ranges (colors) and sizes varying from 0.01 to 2 microns. The availability of carboxylate modified and streptavidin coated beads offers many alternatives for binding them to DNA molecules.

Full	Title	Citation	Front	Review	Classification	Date	Reference	KWIC	Draw Desc	Image
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☐ 50. Document ID: US 5681745 A

L3: Entry 50 of 84

File: USPT

Oct 28, 1997

DOCUMENT-IDENTIFIER: US 5681745 A

TITLE: Biotin-binding containment systems

DEPR:

The full-length streptavidin monomer is 159 amino acids in length, some 30 residues longer than avidin. It contains no carbohydrate and has a slightly acidic isoelectric point of about 6.0 which accounts, in part, for the low non-specific binding level. Each subunit of streptavidin is initially synthesized as a precursor of 18,000 daltons which forms a tetramer of about 75,000 daltons. Secretion and post-secretory processing results in mature subunits having an apparent size of 14,000 daltons. Processing occurs at both the amino and carboxyl termini to produce a core protein of about 13,500 daltons, having about 125 to 127 amino acids. This core streptavidin forms tetramers and binds to biotin as efficiently as natural streptavidin. The mature streptavidin tetramer binds one molecule of biotin per subunit and the complex, once formed, is unaffected by most extremes of pH, organic solvents and denaturing conditions. Separation of streptavidin from biotin requires conditions, such as 8M guanidine, pH 1.5, or autoclaving at 121.degree. C. for 10 minutes. Mutations of the streptavidin or core streptavidin protein exist whereby binding affinity is reduced such that dissociation can be more easily performed without damage to the attached biotin-bound molecule.

DEPR:

One embodiment of the invention is directed to a genetic containment system containing at least one nucleic acid cassette that encodes a biotin-binding component. The component may be used as the suicide function or as a marker for subsequent detection of recombinant organisms. The biotin-binding component is preferably a protein which specifically binds to biotin such as streptavidin or avidin, derivatives or mutations of streptavidin or avidin, or combinations of these components. Biotin-binding components may also be nucleic acids such as RNA, DNA or even PNA sequences that have an affinity for biotin and can be expressed or otherwise obtained from nucleic acid.

CT.PR.

3. The containment system of claim 2 wherein the streptavidin mutation is a core streptavidin protein.

Full	Title	Citation	Front	Review	Classification	Date	Reference	KWIC	Draw Desc	Image
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Terms	Documents
streptavidin near(mutat\$6 or alter\$6 or modif\$6 or mutant\$2)	84

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Display Format: KWIC Change Format

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Generate Collection

Search Results - Record(s) 51 through 84 of 84 returned.☐ 51. Document ID: US 5679533 A

L3: Entry 51 of 84

File: USPT

Oct 21, 1997

DOCUMENT-IDENTIFIER: US 5679533 A

TITLE: Biotin-binding containment systems

DEPR:

The full-length streptavidin monomer is 159 amino acids in length, some 30 residues longer than avidin. It contains no carbohydrate and has a slightly acidic isoelectric point of about 6.0 which accounts, in part, for the low non-specific binding level. Each subunit of streptavidin is initially synthesized as a precursor of 18,000 daltons which forms a tetramer of about 75,000 daltons. Secretion and post-secretory processing results in mature subunits having an apparent size of 14,000 daltons. Processing occurs at both the amino and carboxyl termini to produce a core protein of about 13,500 daltons, having about 125 to 127 amino acids. This core streptavidin forms tetramers and binds to biotin as efficiently as natural streptavidin. The mature streptavidin tetramer binds one molecule of biotin per subunit and the complex, once formed, is unaffected by most extremes of pH, organic solvents and denaturing conditions. Separation of streptavidin from biotin requires conditions, such as 8M guanidine, pH 1.5, or autoclaving at 121.degree. C. for 10 minutes. Mutations of the streptavidin or core streptavidin protein exist whereby binding affinity is reduced such that dissociation can be more easily performed without damage to the attached biotin-bound molecule.

DEPR:

One embodiment of the invention is directed to a genetic containment system containing at least one nucleic acid cassette that encodes a biotin-binding component. The component may be used as the suicide function or as a marker for subsequent detection of recombinant organisms. The biotin-binding component is preferably a protein which specifically binds to biotin such as streptavidin or avidin, derivatives or mutations of streptavidin or avidin, or combinations of these components. Biotin-binding components may also be nucleic acids such as RNA, DNA or even PNA sequences that have an affinity for biotin and can be expressed or otherwise obtained from nucleic acid.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 52. Document ID: US 5679519 A

L3: Entry 52 of 84

File: USPT

Oct 21, 1997

DOCUMENT-IDENTIFIER: US 5679519 A

TITLE: Multi-label complex for enhanced sensitivity in
electrochemiluminescence assay

DEPR:

The beads also include a coating or other means for affixing the oligonucleotide capture probe to the bead. For example, the bead may be coated with streptavidin so as to bind and carry the oligonucleotide probe, the latter being biotinylated for reaction with the streptavidin. Alternatively, the bead may be biotinylated and the probe modified with streptavidin for attachment to the bead. Other alternatives or modifications are contemplated provided the result comprises a magnetic bead carrying an oligonucleotide probe for ECL use.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 53. Document ID: US 5672691 A

L3: Entry 53 of 84

File: USPT

Sep 30, 1997

DOCUMENT-IDENTIFIER: US 5672691 A

TITLE: Recombinant core streptavidin

BSPR:

The object of the present invention was to provide a homogeneous streptavidin mutant protein (core streptavidin) in which the disadvantages of the prior art are at least partially eliminated. A further object of the present invention was the development of a reliable, simple, reproducible and economic production process for a homogeneous core streptavidin which is suitable for the various analytical and preparative applications and in particular for diagnostic tests.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 54. Document ID: US 5658745 A

L3: Entry 54 of 84

File: USPT

Aug 19, 1997

DOCUMENT-IDENTIFIER: US 5658745 A
TITLE: Cell enumeration immunoassay

DEPR:

The cell enumeration immunoassay of the instant invention is a simultaneous solid phase or heterogeneous sandwich immunoassay in which the first labeled antibody, second detectably labeled antibody, modified solid phase and a sample are reacted together. The solid phase or support is modified so that it will bind with the first labeled antibody or capture reagent. Thus, the label on the first antibody is not used for detection but for immobilization onto the solid phase. Typically, the solid phase and first antibody are modified directly or indirectly with members of a specific binding pair which can be immune or non-immune. For example, if the solid phase is modified with streptavidin then the first antibody would be labeled with biotin. Modification of the solid phase is accomplished using techniques well known to those skilled in the art. Similarly, labeling of the first antibody involves techniques well known to those skilled in the art.

Full	Title	Citation	Front	Review	Classification	Date	Reference	KOMIC	Drawn Desc	Image
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☐ 55. Document ID: US 5573909 A

L3: Entry 55 of 84

File: USPT

Nov 12, 1996

DOCUMENT-IDENTIFIER: US 5573909 A
TITLE: Fluorescent labeling using microparticles with controllable stokes shift

DEPR:

Detection of Concanavalin A (Con A) receptors on human lymphocytes in suspension. Human lymphocytes are grown in suspension, fixed with 3.7% formaldehyde in 10 mM sodium phosphate, 0.85% NaCl, pH 7.2 (PBS) and then incubated with biotinylated Con A. Cells are pelleted by centrifugation for 5 minutes at 2000 rpm in a benchtop centrifuge onto microtiter plates and then washed with PBS. A solution containing 3 μ l of 14 nm yellow-green streptavidin-modified carboxylate-modified latex fluorescent microspheres (2% solids) diluted into 40 ml of PBS, 0.2% Tween-20, 3% bovine serum albumin (BSA) is incubated with the cells. Cells are once again pelleted in microtiter plates and washed briefly with PBS containing 0.2% Tween 20 and resuspended in the same solution. An aliquot of labeled cells is removed from each well and applied to a coverslip. Labeled cells are visualized in the fluorescence microscope using a standard fluorescein filter set.

DEPR:

Quantitation of single-stranded M13 bacteriophage DNA using a biotinylated oligonucleotide probe and streptavidin-coated yellow-green fluorescent microspheres. A dilution series of single-stranded M13 DNA (diluted into a 500 ng/ μ l solution of denatured herring sperm DNA in 10 mM Tris, 1 mM EDTA, pH 8.0 (T.E.)), consisting of amounts such as 50 ng, 25 ng, 12.5 ng, 6.3 ng, 3.2 ng, 1.6 ng, 0.8 ng, 0.4 ng and 0 DNA per 1 μ l spot, is spotted onto a nitrocellulose filter membrane that has been pretreated with 3M NaCl, 0.3M sodium citrate, pH 7.0 (20.times. SSC) and air-dried. Samples containing unknown amounts of M13 DNA are prepared according to standard procedures from infected bacterial cells. Fresh M13 plaques are picked using sterile glass capillaries, innoculated into 2 mL of bacterial growth media in sterile glass culture tubes, and allowed to propagate at 37.degree. C., shaking, for 4.5 hours. Bacterial cells and debris are pelleted by centrifugation for 3 minutes at room temperature at 5000 rpm in a benchtop microcentrifuge. 1-2 mL of the

at room temperature at 3000 rpm in a benchtop microcentrifuge. 1.2 ml of the cleared supernatant is transferred to a fresh microfuge tube. A solution containing 20% polyethylene glycol in 2.5M NaCl is added to the supernatant, the tube mixed well by inversion and allowed to stand for 15 minutes at room temperature to allow the phage precipitate to form. The phage are pelleted by centrifugation at 12,000 in a tabletop microcentrifuge for 5 minutes, at 4.degree. C. The supernate is removed and discarded. The pellet is resuspended in 100 .mu.L T.E., then extracted with 50 .mu.L phenol and nucleic acids are precipitated with ethanol and sodium acetate. Nucleic acids are resuspended in a final volume of 50 .mu.L T.E.) Samples containing 1 .mu.L of a 1:10-1:100 dilution of each unknown are then spotted on the filter membrane. The filter is baked at 80.degree. C. in vacuo for 1 hour, prehybridized and hybridized to an appropriate singly biotinylated synthetic oligonucleotide probe, according to standard methods. The filter is washed briefly with 20.times. SSC at room temperature to remove unhybridized probe, blocked again with PBS containing 3% BSA for 30 minutes, and incubated with a solution containing 1 .mu.m streptavidin-modified yellow-green fluorescent carboxylate-modified latex microspheres (0.2% solids final concentration). The filter membrane is then exposed to ultraviolet light at 300 nm and photographed using Polaroid black and white 667 print film, using a Kodak No. 15 or similar Wratten gelatin filter. The intensity of the spot containing the unknown sample is compared to the intensities of dilutions containing known amounts of M13 DNA, in order to determine the amount of DNA in the unknown sample.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KMJC	Drawn Desc	Image
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☐ 56. Document ID: US 5547932 A

L3: Entry 56 of 84

File: USPT

Aug 20, 1996

DOCUMENT-IDENTIFIER: US 5547932 A

TITLE: Composition for introducing nucleic acid complexes into higher eucaryotic cells

BSPR:

The invention also relates to a transfection kit, comprising a carder means having in close confinement therein one or more container means, wherein a first container means contains a biotin-modified endosomolytic agent and a second container means contains a streptavidin-modified substance having affinity for nucleic acid.

DEPR:

The coupling of streptavidin to polylysine was effected using the method described by Wagner et al., 1990, and in EP-A1 388 758. 79 nmol (4.7 mg) of streptavidin in 1 ml of 200 mM HEPES pH 7.9 and 300 mM NaCl were treated with a 15 mM ethanolic solution of SPDP (236 nmol). After 1.5 hours at ambient temperature the modified protein was gel filtered over a Sephadex G-25 column, thereby obtaining 75 nmol of streptavidin, modified with 196 nmol of dithiopyridine linker. The modified protein was reacted under an argon atmosphere with 3-mercaptopropionate-modified polylysine (75 nmol, average chain length 290 lysine monomers, modified with 190 nmol mercapto-propionate linker) in 2.6 ml of 100 mM HEPES pH 7.9, 150 mM NaCl. Conjugates were isolated by cation exchange chromatography on a Mono S HR5 column (Pharmacia). (Gradient: 20-100% buffer B. Buffer A: 50 mM HEPES pH 7.9; buffer B: buffer A plus 3M sodium chloride). The product fraction eluted at a salt concentration of between 1.2M and 1.7M. Dialysis against HBS (20 mM HEPES pH 7.3, 150 mM NaCl) resulted in a conjugate consisting of 45 nmol of streptavidin and 53 nmol of polylysine.

DEPR:

For formation of adenovirus-DNA-transferrin complexes 50 .mu.l of biotinylated adenovirus were incubated with 400 ng of streptavidin-modified polylysine in 20 .mu.l HBS for 20 min. Then 20 .mu.l of HBS containing 6 .mu.g pCMVL were added. After an incubation period for 20 min 7 .mu.g of mouse transferrin-polylysine conjugate (mTfpL) in 160 .mu.l HBS were added and the whole mixture was incubated for further 20 min.

DEPR:

The complexes were prepared as follows: 200 .mu.l biotinylated Adenovirus dl312 diluted with 200 .mu.l HBS were incubated with 6.4 .mu.g streptavidin-modified polylysine in 400 .mu.l HBS for 30 minutes at room temperature. Then 48 .mu.g of pCMV-L in 800 .mu.l HBS were added. After 30 minutes of incubation 48 .mu.g of TfpL in 900 .mu.l HBS were further added. For application of the complexes male Sprague Dawley rats (250 g body weight) were anesthetized with Avertin and the abdomen opened with a median incision. The intestine was displaced to the left side of the body and a 27 G needle, which had been attached to a tube and a 1 ml syringe was inserted into the bile duct. The injection of the complexes was performed over a period of 4 minutes. Then the needle was retracted from the bile duct and the injection site sealed with a fibrin sealer (Immuno). The abdominal wound was closed with sutures and metal clips. After 30 hours the rat was killed and samples from different lobes of the liver were assayed for luciferase gene expression. The peak activity of luciferase was 19000 light units/mg protein and the calculated overall expression in the total liver was in the range of 2.7.times.10.sup.6 light units.

DEPR:

Human skin biopsies were put into a 6 cm petri dish containing DMEM, 2 mM glutamine, 20% FCS and antibiotics. Then the biopsies were thoroughly minced with a surgical knife and cultured in the presence of 3 ml medium for 5 days. Thereafter the cells were washed with fresh DMEM containing 2 mM glutamine 10% FCS and antibiotics and cultured for further 7 days. After this period of time the cells were trypsinized and subcultured into new petri dishes. When the cells were almost confluent, they were trypsinized again and stored frozen until transfection. For transfection the cells were thawed and seeded into 6 cm petri dishes and cultured in DMEM containing 2 mM glutamine 10% FCS and antibiotics. The transfection conjugates were prepared as follows: 3 .mu.l, 10 .mu.l, 20 .mu.l and 30 .mu.l of biotinylated adenovirus dl312 were incubated with 0.1 .mu.g, 0.3 .mu.g, 0.5 .mu.g and 0.8 .mu.g polylysine-modified streptavidin in 150 .mu.l HBS for 30 minutes at room temperature. Then 6 .mu.g of pCMV-.beta.gal plasmid in 170 .mu.l HBS were added and the mixture was incubated for further 30 minutes. In the final step 7.8 .mu.g TfpL for the conjugates with 3 .mu.l dl3 12, 7 .mu.g TfpL for 10 .mu.l dl3 12 and 6 .mu.g TfpL for the conjugates with 20 .mu.l and 30 .mu.l dl312 in 170 .mu.g HBS were added. After an incubation period of 30 minutes the conjugates were applied to the cells in 2 ml DMEM containing 2 mM glutamine, 2% FCS and antibiotics and the cells were incubated for 4 hours at 37.degree. C. Then the medium was removed and culture was continued at 37.degree. C. with DMEM containing 2 mM glutamine, 10% FCS and antibiotics. After 48 hours the expression of .beta.-galactosidase was demonstrated as described in previous Examples.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KM/C	Drawn Desc	Image
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☐ 57. Document ID: US 5489528 A

L3: Entry 57 of 84

File: USPT

Feb 6, 1996

DOCUMENT-IDENTIFIER: US 5489528 A
TITLE: Recombinant core-streptavidin

BSPR:

The object of the present invention was to provide a homogeneous streptavidin mutant protein (core streptavidin) in which the disadvantages of the prior art are at least partially eliminated. A further object of the present invention was the development of a reliable, simple, reproducible and economic production process for a homogeneous core streptavidin which is suitable for the various analytical and preparative applications and in particular for diagnostic tests.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 58. Document ID: US 5482867 A

L3: Entry 58 of 84

File: USPT

Jan 9, 1996

DOCUMENT-IDENTIFIER: US 5482867 A
TITLE: Spatially-addressable immobilization of anti-ligands on surfaces

DRPR:

FIG. 5 presents fluorescence results showing the spatial immobilization of Fluorescein-Biotin on a surface modified with Streptavidin.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 59. Document ID: US 5482836 A

L3: Entry 59 of 84

File: USPT

Jan 9, 1996

DOCUMENT-IDENTIFIER: US 5482836 A
TITLE: DNA purification by triplex-affinity capture and affinity capture electrophoresis

DEPR:

As previously stated, one of the preferred embodiments of the affinity capture electrophoresis method of the invention uses a procedure very similar to the triplex-affinity capture method described above. In the TAC electrophoresis method, the target DNAs, the coupled oligonucleotide probes, oligonucleotide backbone analogs such as polyamide nucleic acids and phosphotriesters, the molecular recognition systems and the solid carriers are the ones used and discussed above for the TAC method. However, coupled ribonucleotides or coupled polynucleotides can also be used to form a triplex or a triplex equivalent and capture intact DNA. Except for reasons which are obvious, solid carriers such as discs, tubes, microplates, etc., are not acceptable. Again, preferably the target DNA contains a double stranded homopurine-homopyrimidine helix. But, again, as discussed above, other types of DNA may also be used. The preferred specific binding partner is a pyrimidine-rich oligonucleotide probe, preferably (T-C).sub.n but other probes including a purine rich oligonucleotide can also be used. The oligonucleotide or other specific binding partner is coupled to a first recognition molecule, preferably biotin

or streptavidin. Again, however, the specific binding partner may also be coupled to molecular recognition molecules belonging to other systems including an antigen/antibody, a protein A/Ig, or a lectin/carbohydrate system. The specific binding partner may be coupled either directly or indirectly to the recognition molecule but, again, care must be taken with indirect coupling to avoid steric hindrance. A preferred specific binding partner is a 5'-biotinylated homopyrimidine oligonucleotide. The sample and the biotinylated oligonucleotide are incubated in a mildly acidic buffer such as sodium acetate buffer (preferably pH approximately 4.5-5.0) but other buffers such as sodium citrate or sodium phosphate having pHs ranging from about 3.5 to about 6.5 as described above may also be used to form the intermolecular triple-helices. Following triplex formation, the mixture is electrophoresed in the gel containing the trap. The trap is a solid carrier preferably uniform glass, plastic or metal beads or particles but other solid phases may also be used providing they are large enough to stay embedded in the gel, will not migrate through the pores of the gel and can be separated from the gel following electrophoresis. A second recognition molecule which corresponds to the first recognition molecule coupled to the probe is attached to the solid carrier to form the solid phase trap. Again, the recognition molecule may be directly or indirectly attached to the solid carrier but care must be taken to prevent interference with the migration of the undesired molecules as well as to prevent steric hindrance. Preferably, the trap is streptavidin uniform coated beads. This trap is preferably prepared by allowing a suspension of beads fixed to the appropriate recognition molecules to solidify in a second well which is placed in front of the origin well of the same lane. In general, the exact amount of the solid phase and the percentage of gel material in the suspension used to prepare the trap will depend upon the amount of material being electrophoresed as well as the percentage of actual gel material (i.e., agarose) in the entire gel. However, in general the percentage of the gel material in the trap will be slightly higher than the percentage of gel material in the entire gel. For example, in an 0.8% agarose gel (0.8%, LE agarose, FMC), an appropriate trap, approximately 1 millimeter thick, is made by allowing a suspension (approximately 5 mgs/ml final concentration) of streptavidin-coated magnetic beads (Dynabeads, streptavidin M-280, Dynal) in 1% molten Inert Agarose (FMC) to solidify. Care must also be taken to maintain the pH of the gel during electrophoresis at a pH which is stable for triplex maintenance. When the TAC method is used in conjunction with affinity capture electrophoresis and the molecular recognition system is a streptavidin, avidin/biotin system, the mixture containing the triple-helices should be electrophoresed in a high ionic strength buffer to minimize non-specific interaction between DNA and streptavidin at acid pH. An alternative solution to the non-specific interaction problem is the use of modified streptavidin. For example, succinylation of the streptavidin-beads reduced the non-specific interaction with DNA at acidic pH, although not completely (data not shown). It would be possible to prepare streptavidin with an even more acidic isoelectric point by site-directed mutagenesis using a recently-developed expression system for this protein (Sano, T. and Cantor, C. R. (1990) Proc. Natl. Acad. Sci. USA 87:142-146). Preferably, electrophoresis is done under cold conditions (about 4.degree. C.) but the temperature may range from about 4.degree. C. to about 40.degree. C. Following electrophoresis, the trap can be removed and treated with alkaline buffer such as 1.0 molar Tris.multidot.HCl, pH 9/0.5 mM EDTA for a time sufficient to break the Hoogsteen hydrogen bonds between the double-helix DNA and the homopyrimidine probe but not the Watson-Crick bonds between the double-helical DNA. Other buffers which can be used to separate the DNA from the solid phase are TE (10 mM Tris-HCl(pH8), 10 mM EDTA or other similar buffers with pHs ranging from about 8.0 to about 13.0, preferably 8.0 to 10.0. If the probe (i.e., specific binding partner) is a ribooligonucleotide, the pH of the buffer should be no higher than about 8.5. The incubation period used for separating the DNA will depend on the reagent used. When Tris.multidot.HCl is used, the time for incubation can vary from about 5 minutes to 2 hours but 20 minutes is usually sufficient. The separated target DNA can then be recovered from the eluate by standard means such as 1:1 (vol/vol) phenol/chloroform extraction and ethanol precipitation.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KMJC	Draw Desc	Image
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☐ 60. Document ID: US 5451683 A

L3: Entry 60 of 84

File: USPT

Sep 19, 1995

DOCUMENT-IDENTIFIER: US 5451683 A

TITLE: Spatially-addressable immobilization of anti-ligands on surfaces

DRPR:

FIG. 5 presents fluorescence results showing the spatial immobilization of Fluorescein-Biotin on a surface modified with Streptavidin.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KMJC	Draw Desc	Image
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☐ 61. Document ID: US 5399331 A

L3: Entry 61 of 84

File: USPT

Mar 21, 1995

DOCUMENT-IDENTIFIER: US 5399331 A

TITLE: Method for protein-liposome coupling

DEPR:

Streptavidin (5 mg/ml in 25 mM HEPES, 150 mM NaCl, pH 7.5; HBS pH 7.5), was modified with the amine reactive reagent, SPDP according to the published procedures of Carlsson, et al, Biochem. J., 173, 723 (1978). Briefly, SPDP (25 mM in methanol) was incubated at a 10 molar ratio to streptavidin at room temperature for 30 minutes. Unreacted SPDP was removed by gel filtration on sephadex G-50 equilibrated with HBS pH 7.5. PDP-modified streptavidin was reduced with DTT (25 mM, 10 minutes). The thiolated product was isolated by gel exclusion on sephadex G-50 equilibrated with the relevant buffer and was immediately used in coupling experiments. The extent of modification of streptavidin was determined by estimating the concentration of the protein at 280 nm (extinction coefficient, E.sub.280 :2770) prior to the addition of dithiothreitol (DTT) and the 2-thiopyridone concentration at 343 nm (E.sub.343 :7550) 10 minutes after addition of DTT. In the case of IgG, after modification with SPDP as described for streptavidin, the protein was fluorescently labelled with FITC-cellite (50% weight of IgG, 20 minutes). Prior to the treatment of the protein with DTT, the sample was separated from unreacted reagents on sephadex G-50 equilibrated with an acetate buffer (100 mM NaCl, 100 mM sodium acetate, pH 5.0) to protect against the reduction of the intrinsic disulfides of the molecule. Both protein preparations were modified to the same extent with SPDP (about 5-6 SPDP molecules per protein).

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KMJC	Draw Desc	Image
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☐ 62. Document ID: US 5374524 A

L3: Entry 62 of 84

File: USPT

Dec 20, 1994

DOCUMENT-IDENTIFIER: US 5374524 A

TITLE: Solution sandwich hybridization, capture and detection of amplified nucleic acids

BSPR:

A further variation has been described in German Preliminary Published Application 3,546,312 A1. This method, like that described by Ranki et al., employs a capture probe and a reporter probe which hybridize to distinct portions of the target nucleic acid. The target nucleic acid is contacted in solution by the two probes. The first, or capture probe, contains a binding component, such as biotin, that is capable of binding with a receptor component, such as streptavidin, which has been affixed to a solid support. After formation of the capture probe--target nucleic acid--reporter probe complex, a streptavidin-modified solid support is added. Any unhybridized reporter probe is washed away followed by the detection of the label incorporated into the complex bound to the solid support. An advantage of this technique over that disclosed by Ranki et al. is that the hybridization, which takes place in solution, is favored kinetically. Some disadvantages are that the length of the target nucleic acid affects the overall efficiency of the reaction which decreases with increasing target nucleic acid length. Also, sandwich nucleic acid probe assays, whether heterogeneous two-step or one-step, or utilizing solution hybridization, are not as sensitive as the direct assay method.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KMIC	Draw Desc	Image
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☐ 63. Document ID: US 5372930 A

L3: Entry 63 of 84

File: USPT

Dec 13, 1994

DOCUMENT-IDENTIFIER: US 5372930 A

TITLE: Sensor for ultra-low concentration molecular recognition

DEPC:

Detection of Biotin in a Sensor Having a Substrate Modified with Biotin and a Tip Modified with Streptavidin

DEPC:

Detection of Biotin in a Sensor Having a Substrate and a Tip Modified with Streptavidin

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KMIC	Draw Desc	Image
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☐ 64. Document ID: US 5252743 A

L3: Entry 64 of 84

File: USPT

Oct 12, 1993

DOCUMENT-IDENTIFIER: US 5252743 A

TITLE: Spatially-addressable immobilization of anti-ligands on surfaces

DRPR:

FIG. 5 presents fluorescence results showing the spatial immobilization of Fluorescein-Biotin on a surface modified with Streptavidin.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 65. Document ID: US 5171578 A

L3: Entry 65 of 84

File: USPT

Dec 15, 1992

DOCUMENT-IDENTIFIER: US 5171578 A

TITLE: Composition for targeting, storing and loading of liposomes

DEPR:

IgGs and streptavidin were modified with the amine reactive heterobifunctional reagent SPDP according to Leserman et al. (supra.). Biotin-conjugated antibodies were prepared according to Bayer et al., Biochim et Biophys Acta, 550, p. 464-473, (1979).

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 66. Document ID: US 5047245 A

L3: Entry 66 of 84

File: USPT

Sep 10, 1991

DOCUMENT-IDENTIFIER: US 5047245 A

TITLE: Novel composition for targeting, storing and loading of liposomes

DEPR:

IgGs and streptavidin were modified with the amine reactive heterobifunctional reagent SPDP according to Leserman et al. (supra.). Biotin-conjugated antibodies were prepared according to Bayer et al., Biochim et Biophys Acta, 550, p. 464-473, (1979).

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 67. Document ID: US 5026785 A

L3: Entry 67 of 84

File: USPT

Jun 25, 1991

DOCUMENT-IDENTIFIER: US 5026785 A

TITLE: Avidin and streptavidin modified water-soluble polymers such as polyacrylamide, and the use thereof in the construction of soluble multivalent macromolecular conjugates

ABPL:

Avidin and streptavidin modified water-soluble polymers are provided, such modified polymers being exemplified by, but not limited to, water-soluble polyacrylamides being substituted by multiple substituents of avidin or streptavidin. The avidin and streptavidin modified polymers provided, may be stored, and later used to bind biotinylated antibodies, biotinylated toxins, or biotinylated isotope-labelled proteins, thus producing homo- or heteroconjugates of known composition. A process for the preparation of certain of the avidin or streptavidin modified polymers and conjugates thereof is also provided.

BSPR:

Attempts to develop reagents for specifically coating tumor cells with antigens that might sensitize them to immune cytotoxicity has led us to the use of water-soluble polymer-protein conjugates, and more specifically, to the use of substantially linear polyacrylamide-based polymer-protein conjugates (polygens). By coupling substantially linear polyacrylamide (PA) with streptavidin (SA) or avidin (A), the convenient general reagents avidin modified polyacrylamide, of which polyacrylamide-avidin (PAA) is a type; and streptavidin modified polyacrylamide, of which polyacrylamide-streptavidin (PASA) is a type have been provided herein. Any of the above avidin or streptavidin modified polymers, provided herein, may be used to prepare multivalent macromolecular conjugates, by coupling any of the same with a wide variety of biotinylated proteins. The preparation of such macromolecular conjugates is based upon a known interaction between biotin and avidin (Bayer and Wilchek, 1980).

BSPR:

With the utilization of PASA or PAA, provided herein, we have prepared water-soluble multivalent heterologating antibody conjugates capable of binding cells of different MHC haplotypes, as well as heteroconjugates of anti-Class I MHC antibodies and soluble Class I MHC molecules; these conjugates have been used to coat murine tumor cells with allogeneic Class I MHC molecules. The same procedure has the potential for, and is fully envisioned as, being useful in the preparation of a wide range of "off the shelf" ad hoc multivalent conjugates. Such multivalent conjugates could be either immunotoxins, tumor labeling reagents, or vaccines easily prepared by mixing any of a variety of biotinylated antibodies or toxins or isotope-labelled proteins or unlabelled proteins or peptides with any of the avidin or streptavidin modified water-soluble polymers disclosed herein, including avidin modified polyacrylamide, streptavidin modified polyacrylamide, polyacrylamide-streptavidin (PASA) and polyacrylamide-avidin (PAA).

BSPR:

The present invention is also directed to a simplified procedure for making biotinylated protein conjugates with certain avidin and streptavidin modified polymers, disclosed herein, more specifically, avidin modified polyacrylamide, streptavidin modified polyacrylamide, PASA and PAA.

BSPR:

Several features of applicants' process for preparing avidin and streptavidin modified polyacrylamides, including PASA and PAA should be noted. First, in contrast to previous uses of a carbodiimide to link proteins to each other or to polymers, in an aqueous environment, in the present invention activated polyacrylamide is separated from excess carbodiimide prior to adding avidin or streptavidin. This removal of excess carbodiimide prevents intermolecular crosslinking of avidin or streptavidin protein by a carbodiimide. Secondly, in contrast to prior processes, applicants, during the coupling step of avidin or streptavidin to polyacrylamide, utilize excess avidin or streptavidin to inhibit intermolecular crosslinking of polyacrylamide. Thirdly, in contrast to prior processes, by utilizing applicants' processes, once the capacity of avidin modified polyacrylamide, streptavidin modified polyacrylamide, PASA or PAA for biotinylated proteins has been measured, and appropriate biotinylated

proteins prepared, preparation of conjugates requires no further activation, use of crosslinking agents, or purification from unbound protein and crosslinking agent.

BSPR:

1) An avidin or streptavidin modified water-soluble polymer, comprising:

BSPR:

2) An avidin or streptavidin modified water-soluble polyacrylamide, comprising:

BSPR:

3) An avidin or streptavidin modified polyacrylamide, comprising:

BSPR:

4) An avidin or streptavidin modified polyacrylamide, comprising:

BSPR:

5) An avidin or streptavidin modified polyacrylamide, prepared by the process steps of:

BSPR:

(IV) reacting, subsequent to Step (III), said resultant product with an excess of avidin or streptavidin, and thereby forming said avidin or streptavidin modified polyacrylamide in situ; and

BSPR:

(V) separating, subsequent to step (IV), said avidin or streptavidin modified polyacrylamide from excess streptavidin or avidin.

BSPR:

6) Any of the above avidin or streptavidin modified polymers provided herein, in (1) to (5), additionally substituted by a variety of either biotinylated antibodies, or biotinylated toxins, or soluble biotinylated isotope-labeled proteins.

BSPR:

7) A process for the preparation of an avidin or streptavidin modified polyacrylamide, comprising the process steps of:

BSPR:

(IV) reacting, subsequent to step (III), said resultant product with an excess of avidin or streptavidin, and thereby forming said avidin or streptavidin modified polyacrylamide in situ; and

BSPR:

(V) separating, subsequent to step (IV), said avidin or streptavidin modified polyacrylamide from excess streptavidin or avidin.

DEPR:

The present invention provides for products, wherein water-soluble polymers such as water-soluble polyacrylamide have been substituted by either multiple avidin or streptavidin substituents. Methods for preparing such products wherein polyacrylamides are the water-soluble polymers are also provided. Inasmuch as the process for preparing polyacrylamide based products, provided herein, utilizes as raw materials water-soluble polyacrylamides, as well as other starting materials, a discussion of suitable starting materials useful in such a process will be incorporated into the following discussion of process steps utilized to prepare avidin modified polyacrylamides, of which PAA is an example, and, streptavidin modified polyacrylamides, of which PASA is an example.

DEPR:

Finally, again we note that a suitable starting polyacrylamide raw material must be soluble in an aqueous based solution inasmuch as avidin and

streptavidin modified polyacrylamide products produced therefrom, including PASA and PAA, are to be soluble in aqueous based solutions. All polyacrylamides encompassed herein being within the average molecular weight range provided (i.e., between about 50,000 to 2.times.10.sup.6 daltons), and if substantially linear, should readily meet with this requirement. If, however, one finds a polyacrylamide which might otherwise would be a suitable starting polyacrylamide raw material, but which is not completely soluble in 2 parts purified water, USP, on a weight per weight basis at a temperature of about 23.degree. C., the same is expressly excluded herefrom.

DEPR:

As should be apparent from reaction scheme I(b), in order to couple avidin or streptavidin to polyacrylamide by methods provided herein it is necessary that activated carboxyl groups exist on a polyacrylamide backbone. Furthermore, we note that given reaction I(a), above, is reversable, there can exist carboxyl groups (i.e., C(O)OH) on the avidin or streptavidin modified polyacrylamides provided herein. The amount of such carboxyl groups on the avidin or streptavidin modified polyacrylamide ultimately produced, will depend upon the initial quantity of amide groups which are hydrolyzed into carboxy groups (i.e., 1-50%) and of these carboxy groups--how many are ultimately substituted by avidin or streptavidin, through the coupling process provided herein.

DEPR:

We note also that techniques are available to those skilled in the art to convert carboxyl groups, contained on a streptavidin or avidin modified polyacrylamide as provided herein, into amide groups. The use of such techniques should be readily apparent to those skilled in the art. For example, sulfonyl chloride may be utilized to convert carboxyl groups into acid chlorides, which are then reacted with ammonia to form amides. Use of such a technique is considered fully encompassed herein, and moreover, resulting avidin or streptavidin modified polyacrylamides produced utilizing such techniques are considered fully encompassed herein. Furthermore, due to the availability of such techniques, avidin and streptavidin modified polyacrylamide products not containing carboxyl groups are considered encompassed herein.

DEPR:

Once an avidin or streptavidin modified polyacrylamide has been produced in situ, it is separated from the reaction mixture. Such separations, as performed herein, utilized size exclusion chromatography, and the same is illustrated in the Experimental section contained herein. The use of such a chromatographic method is not considered limiting to the present invention, however, since other means of separation readily apparent to those skilled in the art could be utilized, without departing from the spirit and scope of the present invention. The only requirement for an appropriate separation technique is that it not be too unduly difficult, or produce unsatisfactory results.

DEPR:

Once step V has been completed, the resultant avidin or streptavidin modified polyacrylamide can be stored and later utilized as a chemical reagent to prepare any of a variety of multivalent protein-protein conjugates. For example, streptavidin modified polyacrylamides, provided herein, have been stored for up to four (4) months at about 0.degree. C., and shown to remain stable. The use of any of the avidin or streptavidin polyacrylamides, provided herein, to form multivalent protein-protein conjugates after storing is considered encompassed herein. Moreover, we consider this to be one of the preferred embodiments of the present invention, inasmuch as such a method allows for the "ad hoc" or "off the shelf" preparation of an endless variety of multivalent protein-protein conjugates.

DEPR:

The above "ad hoc" or "off the shelf" method of preparation for an endless variety of multivalent protein-protein conjugates, would of course also be applicable to those avidin or streptavidin modified water-soluble polymers

other than avidin and streptavidin modified polyacrylamides which are provided herein.

DEPR:

It is expected that utilization of the above "ad hoc" technique will allow those skilled in the art to efficiently and easily prepare multivalent conjugates with a variety of either biotinylated antibodies, biotinylated toxins, or biotinylated isotope labelled or unlabelled proteins. Moreover, such varieties of biotinylated antibodies, toxins, or isotope-labelled or unlabelled proteins, can be coupled to the avidin or streptavidin modified water-soluble polymers, provided herein, in conceivably any proportions or ratios desired.

DEPR:

These results show that polyacrylamide-streptavidin (PASA) can be used with biotinylated antibodies and antigens to construct soluble multivalent macromolecular conjugates that can specifically coat tumor cells with MHC antigens of a different haplotype. Furthermore, these results are illustrative of the general utility of either avidin or streptavidin modified water-soluble polymers such as polyacrylamide, as provided herein, in the construction of multivalent protein-protein conjugates.

CLPR:

1. A water-soluble avidin or streptavidin modified polyacrylamide, prepared by the process steps of:

CLPR:

2. The avidin or streptavidin modified polyacrylamide of claim 1, wherein:

CLPR:

3. The modified polyacrylamide of claim 2, wherein said modified polyacrylamide is a streptavidin modified polyacrylamide.

CLPR:

4. The avidin or streptavidin modified polyacrylamide of claim 1, additionally substituted, subsequent to step (V), by a variety of biotinylated antibodies, or biotinylated toxins, or biotinylated isotope-labelled proteins.

CLPR:

5. The avidin or streptavidin modified polyacrylamide of claim 2, additionally substituted, subsequent to step (V), by a variety of biotinylated antibodies, or biotinylated toxins, or biotinylated isotope-labelled proteins.

CLPR:

6. The avidin or streptavidin modified polyacrylamide of claim 3, additionally substituted, subsequent to step (V), by a variety of biotinylated antibodies, or biotinylated toxins, or biotinylated isotope-labelled proteins.

CLPV:

(IV) reacting, subsequent to step (III), said resultant product with an excess of avidin or streptavidin, and thereby forming said avidin or streptavidin modified polyacrylamide in situ; and

CLPV:

(V) separating, subsequent to step (IV), said avidin or streptavidin modified from excess streptavidin or avidin.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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RWMC	Draw Desc	Image
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L3: Entry 68 of 84

File: USPT

Dec 5, 1989

DOCUMENT-IDENTIFIER: US 4885172 A

TITLE: Composition for targeting, storing and loading of liposomes

DEPR:

IgGs and streptavidin were modified with the amine reactive heterobifunctional reagent SPDP according to Leserman et al. (supra.). Biotin-conjugated antibodies were prepared according to Bayer et al., Biochim et Biophys Acta, 550, p. 464-473, (1979).

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KM/C	Draw Desc	Image
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☐ 69. Document ID: US 5846537 A

L3: Entry 69 of 84

File: EPAB

Dec 8, 1998

DOCUMENT-IDENTIFIER: US 5846537 A

TITLE: Modified avidin and streptavidin and methods of use thereof

FPAR:

The present invention provides modified avidin and streptavidin compounds that have suitable blood clearance kinetics for use in a two-step approach to deliver a molecule to a target site. In particular, to hasten SA's blood clearance carbohydrate moieties are covalently bonded to SA. To prolong Avid's blood clearance, Avid is deglycosylated and/or neutralized by alkylation of its lysine amino acids. In a two-step approach, biotinylated compounds are used to deliver radionuclides, cytotoxic drugs, MRI agents, fluorochromes and other agents suitable for imaging and therapy to target-bound modified streptavidin or avidin conjugated antibodies or other targeting agents.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KM/C	Draw Desc	Image
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☐ 70. Document ID: WO 9840396 A1

L3: Entry 70 of 84

File: EPAB

Sep 17, 1998

DOCUMENT-IDENTIFIER: WO 9840396 A1

TITLE: MULTIFLAVOR STREPTAVIDIN

FPAR:

Compounds and methods are described for producing streptavidin mutants with changed affinities. In particular, modifications to the sequence of the natural streptavidin gene is described to create amino acid substitutions resulting in greater affinity for biotin substitutes than for biotin.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KM/C	Draw Desc	Image
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☐ 71. Document ID: EP 799890 A2

L3: Entry 71 of 84

File: EPAB

Oct 8, 1997

DOCUMENT-IDENTIFIER: EP 799890 A2

TITLE: Recombinant inactive core streptavidin mutants

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 72. Document ID: WO 9711183 A1

L3: Entry 72 of 84

File: EPAB

Mar 27, 1997

DOCUMENT-IDENTIFIER: WO 9711183 A1

TITLE: STREPTAVIDIN MUTANTS

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 73. Document ID: WO 9640761 A1

L3: Entry 73 of 84

File: EPAB

Dec 19, 1996

DOCUMENT-IDENTIFIER: WO 9640761 A1

TITLE: MODIFIED AVIDIN AND STREPTAVIDIN AND METHODS OF USE THEREOF

FPAR:

The present invention provides modified avidin and streptavidin compounds that have suitable blood clearance kinetics for use in a two-step approach to deliver a molecule to a target site. In particular, to hasten SA's blood clearance carbohydrate moieties are covalently bonded to SA. To prolong Avid's blood clearance, Avid is deglycosylated and/or neutralized by alkylation of its lysine amino acids. In a two-step approach, biotinylated compounds are used to deliver radionuclides, cytotoxic drugs, MRI agents, fluorochromes and other agents suitable for imaging and therapy to target-bound modified streptavidin or avidin conjugated antibodies or other targeting agents.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 74. Document ID: WO 9624606 A1

L3: Entry 74 of 84

File: EPAB

Aug 15, 1996

DOCUMENT-IDENTIFIER: WO 9624606 A1
TITLE: MODIFIED-AFFINITY STREPTAVIDIN

FPAR:

CHG DATE=19990617 STATUS=O>Streptavidin tetramers have at least one monomer containing an amino acid modification that produces a reduced binding affinity for biotin, a modified off-rate, a modified on-rate, or an altered binding enthalpy. Polynucleotides encoding the modified monomers are also provided. The modified streptavidin and chimeric streptavidin molecules are useful in methods of bioseparations and cell sorting, imaging, drug delivery, and diagnostics.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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RWC	Draw Desc	Image
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☐ 75. Document ID: AU 200066071 A, WO 200107084 A1

L3: Entry 75 of 84

File: DWPI

Feb 13, 2001

DERWENT-ACC-NO: 2001-182731
DERWENT-WEEK: 200128
COPYRIGHT 2001 DERWENT INFORMATION LTD

TITLE: New anti-growth factor receptor avidin fusion proteins comprising a first segment and a second segment useful as a universal drug delivery system and for in vitro and in vivo gene transfer into tumor cells or cells with genetic defects

ABTX:

(b) a segment comprising a protein domain that is an avidin, an avidin mutein, a chemically modified avidin derivative, streptavidin, a streptavidin mutein, or a chemically modified streptavidin derivative.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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RWC	Draw Desc	Image
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☐ 76. Document ID: AU 200063441 A, WO 200105977 A1

L3: Entry 76 of 84

File: DWPI

Feb 5, 2001

DERWENT-ACC-NO: 2001-147344
DERWENT-WEEK: 200128
COPYRIGHT 2001 DERWENT INFORMATION LTD

TITLE: Avidin and streptavidin mutants comprising tryptophan 110 and tryptophan 120 respectively, substituted by lysine for use in all known avidin/streptavidin-biotin systems and a purification process

ABTX:

NOVELTY - A mutant avidin (avidin-W110K) (I) in which tryptophan 110 is substituted by lysine, or mutant streptavidin (Savm-W120K) (II) in which tryptophan 120 is substituted by lysine, is new.

TTX:

AVIDIN STREPTAVIDIN MUTANT COMPRISE TRYPTOPHAN TRYPTOPHAN RESPECTIVE
SUBSTITUTE LYSINE AVIDIN STREPTAVIDIN BIOTIN SYSTEM PURIFICATION PROCESS

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 77. Document ID: EP 1108015 A1, WO 200011152 A1, AU 9957859 A

L3: Entry 77 of 84

File: DWPI

Jun 20, 2001

DERWENT-ACC-NO: 2000-224689

DERWENT-WEEK: 200135

COPYRIGHT 2001 DERWENT INFORMATION LTD

TITLE: Mutant streptavidin molecule, useful as an adaptor and a coating for substrates such as vascular devices or prostheses, comprises a biotin binding domain and a secondary functional domain

TTX:< br> MUTANT STREPTAVIDIN MOLECULAR USEFUL ADAPT COATING SUBSTRATE
VASCULAR DEVICE PROSTHESIS COMPRISE BIOTIN BIND DOMAIN SECONDARY FUNCTION
DOMAIN

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 78. Document ID: EP 977770 A1, WO 9840396 A1, AU 9867014 A

L3: Entry 78 of 84

File: DWPI

Feb 9, 2000

DERWENT-ACC-NO: 1998-506689

DERWENT-WEEK: 200012

COPYRIGHT 2001 DERWENT INFORMATION LTD

TITLE: Streptavidin mutant with higher affinity for biotin substitute than for biotin - useful in, e.g. biological applications utilising streptavidin-biotin binding when endogenous biotin presence would otherwise preclude this approach

ABTX:

Streptavidin mutant having a higher affinity for a biotin substitute (especially 2-iminobiotin) than for biotin is encoded by a nucleic acid sequence comprising aa residues 16-133 of a 159 aa sequence (S1) (not given in the specification) encoding natural streptavidin, and where the sequence comprises at least 1 codon substitution resulting in at least 1 mutant aa substitution.

TTX:

STREPTAVIDIN MUTANT HIGH AFFINITY BIOTIN SUBSTITUTE BIOTIN USEFUL BIOLOGICAL
APPLY UTILISE STREPTAVIDIN BIOTIN BIND ENDOGENOUS BIOTIN PRESENCE PRECLUDE
APPROACH

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 79. Document ID: US 6103493 A, EP 835934 A2, DE 19641876 A1

L3: Entry 79 of 84

File: DWPI

Aug 15, 2000

DERWENT-ACC-NO: 1998-218868
DERWENT-WEEK: 200041
COPYRIGHT 2001 DERWENT INFORMATION LTD

TITLE: Streptavidin mutants with higher binding affinity for peptide ligands - have mutation in amino acid region 44-53, used to isolate, purify or determine fusion proteins including these ligands

T TX:

STREPTAVIDIN MUTANT HIGH BIND AFFINITY PEPTIDE LIGAND MUTANT AMINO ACID REGION
ISOLATE PURIFICATION DETERMINE FUSE PROTEIN LIGAND

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWMC	Draw Desc	Image
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☐ 80. Document ID: US 6207390 B1, WO 9711183 A1, AU 9659177 A, EP 856055 A1, US 6022951 A

L3: Entry 80 of 84

File: DWPI

Mar 27, 2001

DERWENT-ACC-NO: 1997-202890
DERWENT-WEEK: 200119
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TITLE: New streptavidin mutants - have increased stability or altered affinity for biotin

TTX:

NEW STREPTAVIDIN MUTANT INCREASE STABILISED ALTER AFFINITY BIOTIN

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWMC	Draw Desc	Image
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☐ 81. Document ID: US 5846537 A, WO 9640761 A1, AU 9661091 A, EP 832111 A1

L3: Entry 81 of 84

File: DWPI

Dec 8, 1998

DERWENT-ACC-NO: 1997-065174
DERWENT-WEEK: 199905
COPYRIGHT 2001 DERWENT INFORMATION LTD

TITLE: Streptavidin modified for optimal blood clearance kinetics - by linkage to carbohydrate, opt. esterified, for conjugation with targetting agent for biotinylated therapeutic and imaging agent delivery

TTX:

STREPTAVIDIN MODIFIED OPTIMUM BLOOD CLEARANCE KINETIC LINK CARBOHYDRATE OPTION
ESTERIFICATION CONJUGATE TARGET AGENT BIOTINYLATED THERAPEUTIC IMAGE AGENT
DELIVER

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWMC	Draw Desc	Image
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☐ 82. Document ID: JP 07289264 A

L3: Entry 82 of 84

File: DWPI

Nov 7, 1995

DERWENT-ACC-NO: 1996-015269
DERWENT-WEEK: 199602
COPYRIGHT 2001 DERWENT INFORMATION LTD

TITLE: New mutant streptavidin (SA) gene - fused to a firefly luciferase gene,
for the recombinant prepn. of a SA-FL fused protein

TTX:

**NEW MUTANT STREPTAVIDIN GENE FUSE LUCIFERASE GENE RECOMBINATION PREPARATION
FUSE PROTEIN**

Full	Title	Citation	Front	Review	Classification	Date	Reference
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RMC	Draw Desc	Image
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☐ 83. Document ID: US 6225455 B1, EP 372707 A, CA 2001719 A, JP 02288900 A, EP 372707 B1, DE 68925869 E, ES 2087869 T3, IL 92124 A, US 5986061 A, US 6150503 A

L3: Entry 83 of 84

File: DWPI

May 1, 2001

DERWENT-ACC-NO: 1990-180507
DERWENT-WEEK: 200126
COPYRIGHT 2001 DERWENT INFORMATION LTD

TITLE: Phosphorylated modified proteins, including modified interferon(s) -
used in diagnostic and therapeutic applications. e.g. pharmacokinetic studies
and tumour treatment

ABTX:

USE/ADVANTAGE- (I) are used biological and medical fields including
therapeutic and diagnostic applicns.. Specific applicns. include use in
pharmokinetic studies, as an anticancer therapeutic "bomb" (using streptavidin
modified with multiple phosphorylatable gps.), for improvements in hormone
therapy and for enhancement of immunoassays.

ABEQ:

USE/ADVANTAGE- (I) are used biological and medical fields including
therapeutic and diagnostic applicns.. Specific applicns. include use in
pharmokinetic studies, as an anticancer therapeutic "bomb" (using
streptavidin modified with multiple phosphorylatable gps.), for improvements
in hormone therapy and for enhancement of immunoassays.

ABEQ:

USE/ADVANTAGE- (I) are used biological and medical fields including
therapeutic and diagnostic applicns.. Specific applicns. include use in
pharmokinetic studies, as an anticancer therapeutic "bomb" (using
streptavidin modified with multiple phosphorylatable gps.), for improvements
in hormone therapy and for enhancement of immunoassays.

ABEQ:

USE/ADVANTAGE- (I) are used biological and medical fields including
therapeutic and diagnostic applicns.. Specific applicns. include use in
pharmokinetic studies, as an anticancer therapeutic "bomb" (using
streptavidin modified with multiple phosphorylatable gps.), for improvements
in hormone therapy and for enhancement of immunoassays.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 84. Document ID: WO 8705026 A, AU 8771652 A, EP 258411 A, JP 63502560 W, US 4839293 A

L3: Entry 84 of 84

File: DWPI

Aug 27, 1987

DERWENT-ACC-NO: 1987-250198

DERWENT-WEEK: 198735

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TITLE: DNA encoding streptavidin - obtd. by restriction endo-nuclease digestion of chromosomal DNA of Streptomyces avidinii< br>

ABEQ:

DNA which encodes the formation of the polypeptide streptavidin has been isolated by the action of a restriction endonuclease on the chromosomal DNA of Streptomyces avidinii. The nucleic acid sequence of the gene and the aminoacid sequence of the polypeptide have been determined. The streptavidin gene has been fused with a gene that encodes human light density lipoprotein ("LDL") receptor, and the fused gene encodes a modified streptavidin-LDL receptor polypeptide.

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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84

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